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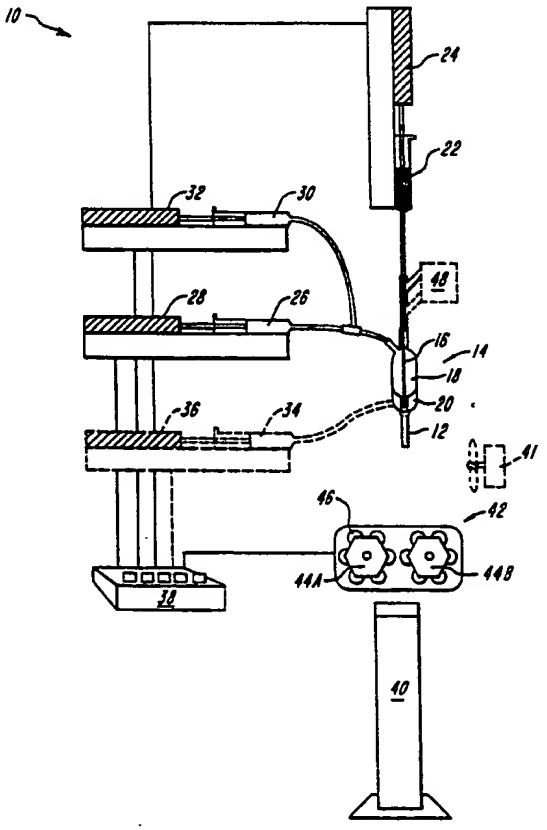
(57) Abstract

Living cells (56) which produce biologically active factors can be encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension (54) and a polymeric solution (52) through a common port (14) to form a tubular extrudate (12) having a polymeric membrane which encapsulates the cell suspension. For example, the cell suspension and the polymeric solution can be extruded through a common extrusion port having at least two concentric bores (16 and 18), such that the cell suspension is extruded through the inner bore (16) and the polymeric solution is extruded through the outer bore (18). The polymeric solution coagulates to form an outer coating or membrane. As the outer membrane is formed, the ends of the tubular extrudate can be sealed to form a cell capsule. In one embodiment, the tubular extrudate is sealed at intervals to define separate cell compartments connected by polymeric links (58). In another embodiment, a cell capsule connected to a tethering filament (59) can be formed.



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CELL CAPSULE EXTRUSION SYSTEMS

Background of the Invention

The technical field of this invention concerns the encapsulation of living cells for the production of biologically active factors.

There is considerable interest at present in the biologically active products of living cells, including, for example, neurotransmitters, hormones, cytokines, nerve growth factors, angiogenesis factors, blood coagulation factors, lymphokines, enzymes and other therapeutic agents. There is also substantial interest in developing new methods and systems for producing such biological factors as well as in delivering these factors to subjects for therapeutic purposes.

For example, Parkinson's disease is characterized by the degeneration of the dopaminergic nigrostriatal system. Striatal implantation of polymer rods which release sustained amounts of a neurotransmitter, dopamine, has been reported to alleviate experimental Parkinsonism in rodents, indicating that the release of dopamine alone in the proper target structure may be able to correct this functional deficiency.

In contrast to the limited capacity of a polymeric matrix drug release system, encapsulated dopamine-releasing cells have been proposed as a means to provide a continuous supply of neurotransmitters. The encapsulation of neurotransmitter-secreting cells by a permselective membrane which permits diffusion of the biological factor may not only prohibit the escape of mitotically active cells, but also prevent host rejection in the case of cross-species transplantation.

A number of researchers have proposed the use of microcapsules -- tiny spheres which encapsulate a microscopic droplet of a cell solution -- for both therapeutic implantation purposes and large scale production of biological products. However, there are a number of shortcomings to the microencapsulation approach: the microcapsules can be extremely difficult to handle (and retrieve, after implantation); their volume is limited; and the types of encapsulating materials which can be used are constrained (by the formation process) to polymers which can dissolve in biocompatible solvents.

An alternative approach has been macroencapsulation, which typically involves loading cells into hollow fibers and then closing the extremities at both ends with a polymer glue. In contrast to microcapsules, macrocapsules offer the advantage of easy retrievability, an important feature in therapeutic (especially, neural) implants. However, the construction of macrocapsules in the past has often been tedious and labor intensive. Moreover, due to unreliable closure, conventional methods of macroencapsulation have provided inconsistent results.

There exists a need for better techniques for macroencapsulation of cells for both therapeutic implantation and industrial production purposes. Encapsulation techniques which can be practiced in a an automated fashion, and which permit the usage of a wider range of materials and/or provide more reliable closure would satisfy a long felt need in the art.

Summary of the Invention

Methods and systems are disclosed for encapsulating viable cells which produce biologically-active factors. The cells are encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension and a polymeric solution through a common port to form a tubular extrudate having a polymeric outer coating which encapsulates the cell suspension.

In one aspect of the invention, methods are disclosed in which the cell suspension and the polymeric solution are extruded through a common extrusion port having at least two concentric bores, such that the cell suspension is extruded through the inner bore and the polymeric solution is extruded through the outer bore. The polymeric solution coagulates to form an outer coating. As the outer coating is formed, the ends of the tubular extrudate can be sealed to form a cell capsule. In one illustrated embodiment, the tubular extrudate is sealed at intervals to define separate cell compartments connected by polymeric links.

Strings of cell capsules formed in this manner have a number of advantages over conventional, cell-encapsulating products. The multi-compartment form ensures that breaks in the tubular membrane can be contained to individual cell capsules. Moreover, the design is particularly advantageous in preparing implantable cell cultures for delivery of

biologically-active factors to a subject for therapeutic purposes. The string of cell capsules can be coiled, twisted or otherwise deposited in various shapes to provide a dense and compact structure for implantation. Because the cell capsules are connected to each other, they can also be readily retrieved, if necessary, following implantation. The string-like nature of these products is particularly preferable over individual spherical microcapsules which typically are retrieved by aspiration (often resulting in a high percentage of unretrievable capsules and, consequently, inflamation in the subject).

Multi-compartment cell capsule strings can be formed from the tubular extrudate of the present invention by sealing the extrudate at intervals using various techniques. For example, the extrudate can be sealed by compressing it at intervals using mechanical or pneumatic force. Alternatively, the pressure under which the cell suspension or the polymeric solution is extruded can be modified to collapse the tubular extrudate at intervals and define separate cell compartments. In yet another technique, the flow of the cell suspension can be interrupted or otherwise impeded at intervals to likewise collapse the tubular extrudate and define cell compartments.

The products of the present invention are particularly well-suited for use and therapeutic implant devices, such as those disclosed in U.S. Patent 4,892,538, "In Vivo Delivery Of Neurotransmitters By Implanted, Encapsulated Cells" by Aebischer et al. issued January 9, 1990, herein incorporated by reference. In U.S. Patent 4,892,538, techniques are disclosed for implanting encapsulated neurotransmitter-secreting cells into a target region within a subject's brain, such that the encapsulated cells secret a neurotransmitter and thereby permit constitutive delivery of a therapeutic agent to treat a neurological deficiency, such as Parkinson's disease. Alternatively, artificial organs capable of secreting other biological factors, such as hormones (e.g., insulin, thymic factors and the like) can also be constructed using the tubular extrudates and multi-compartment cell capsule strings of the present invention.

The cell capsules are also well-suited for use in bioreactors and other in vitro culturing systems, for the production of drugs and other useful biological materials. In such applications, cells which produce such materials, either naturally, by mutation or by recombinant design, are encapsulated and allowed to synthesize the materials which can be collected following secretion into a circulating culture medium. Alternatively, the biological materials can be accumulated within the cell capsules (e.g., by appropriate control of the porosity) and then harvested by removing the strands from the culture medium, lyzing the polymeric membranes and recovering the synthesized materials in concentrated form.

The polymeric coating is preferably a semipermeable membrane, that is to say, a porous structure capable of protecting transplanted cells from autoimmune or viral assault, as well as from other detrimental agents in the external environment, while allowing essential nutrients, cellular waste products and cell secretions to diffuse therethrough. As used herein, the term "selectively permeable" or "semipermeable" is used to describe biocompatible membranes which allow diffusion therethrough of solutes having a molecular weight up to about 150,000 (Mr).

The permeability of the polymeric coating can be varied by controlling the viscosity of the polymeric solution, such that upon coagulation, the coating will form with a network of microchannels to provide diffusion pathways. In one embodiment, this can be achieved by employing a water-miscible solvent as a component of the polymeric solution and maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during extrusion. As the tubular extrudate forms, water from the aqueous cell suspension infiltrates into the coagulating polymer to replace the solvent as the solvent is driven outward by the pressure difference. Upon coagulation, the water which has infiltrated into the polymeric coating provides a network of pores. The optimal pressure and viscosity will, of course, vary with the solvent and polymer employed but can be readily ascertained for any particular polymer/solvent combination by those skilled in the art without undue experimentation.

In another aspect of the invention, systems are disclosed for encapsulating cells to produce the tubular extrudate and multi-compartment cell capsule products described above. This system can include an extrusion head assembly (e.g., a spinneret or the like) having a first inner bore and a second, concentric, outer bore, as well as a cell suspension supply means for supplying the aqueous cell suspension to the inner bore of the extrusion head assembly, and a polymeric solution supply means for supplying the polymeric solution to the outer pore of the extrusion head assembly. As the cell suspension and polymeric solution are co-extruded, they form a tubular extrudate having a polymeric outer coating which encapsulate the cell suspension.

The tubular extrudate can be sealed at intervals by any one of a number of mechanisms. In one illustrated embodiment, two wheels with occluding elements on their periphery cooperate in rotation to periodically pinch the tubular extrudate and thereby seal it. This mechanical compression system can be replaced by a variety of other mechanical or pneumatic compression systems to seal the tubular extrudate at intervals.

Alternatively, the system can include a flow control means for varying the pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion. For example, each of the components supply means can include an infusion pump which is operated by a computer or other control element. In the normal operation, the infusion pumps are controlled to maintain a pressure differential between the aqueous cell suspension and the polymeric solution, such that the polymer solvent is driven outward during coagulation. By periodically varying the pressure, the tubular extrudate can be collapsed at intervals to define individual cell compartments. This can be accomplished, for example, by reducing the aqueous solution pressure. In some instances, it may be preferable to terminate the flow of the aqueous solution entirely and create a vacuum to ensure a complete seal between compartments.

Various other techniques can likewise be employed to interrupt the flow of the aqueous solution at intervals and thereby cause the tubular extrudate to collapse and form multiple compartments. For example, a retraction mechanism can be incorporated into the extrusion head assembly for moving the inner bore relative to the outer bore, such that the flow of the aqueous solution is interrupted to define separate cell compartments at intervals.

The systems disclosed herein can further include a quenchent bath for coagulating the polymeric solution following extrusion, and various mechanisms for drying the tubular extrudate as it emerges from the extrusion head, including blowers, or evacuation chambers. The extrusion head assembly can incorporate additional bores to provide multiple coatings or to deliver a quenchent fluid about the tubular extrudate. The system can also include a sedimentation chamber for the cell suspension, or an equivalent cell packing mechanism, to increase the cell density within the aqueous cell suspension.

The invention will next be described in connection with certain illustrated embodiments; however, it should be clear that various additions, subtractions or modifications can be made by those skilled in the art without departing from the spirit or scope of the invention.

Brief Description of the Drawings

FIG. 1 is an overall schematic diagram of a system for encapsulating viable cells according to the invention;

FIG. 2 is a more detailed schematic diagram of an extrusion head assembly for use in the system of FIG. 1;

FIG. 3 is a schematic diagram of an alternative extrusion head assembly for use in the system of FIG. 1;

FIG. 4 is a schematic diagram of a mechanism for periodically sealing a tubular extrudate according to the invention to form a multi-compartment cell culturing vehicle;

FIG. 5 is a schematic diagram of a mechanism for forming tethered cell capsules;

FIG. 6 is a graph showing dopamine release versus time for capsules containing dopamine secreting cells produced according to the present invention with three different solvent systems;

FIG. 7 is graph showing dopamine release by PC12 cells under normal and potassium-stimulated conditions at various times following encapsulation according to the invention;

FIG. 8A is a graph showing the release of catecholamines from encapsulated PC12 cells; and

FIG. 8B is a graph showing the release of catecholamines from encapsulated chromaffin cells.

Detailed Description

In FIG. 1, a system 10 is shown for producing a tubular extrudate 12 according to the present invention, including an extrusion head 14 having a first (innermost) bore 16, a second outer bore 18 and, optionally, a third (outermost) bore 20. The system 10 further includes a cell suspension supply 22 and an associated pump 24, a polymer solution supply 26 and an associated pump 28 and, optionally, a flush solution supply 30 with a pump 32. Additionally, the system can also, optionally, include a outer flowing quenchent supply 34 with an associated pump 36. All of the pump elements can be controlled manually or, preferably, by an automated controller (e.g., a microprocessor) 38. The system 10 can also include a quenchent bath 40, which would normally be disposed directly below the extrusion head 14 during operation. Alternatively, the system can include a blower 41 or the system can be employed within an evacuated or other reduced pressure chamber to aid in solvent removal.

When the system 10 is employed to shape the tubular extrudate into a multi-compartment cell capsule string, a sealing means can be employed. One such sealing element 42 is shown in FIG. 1, including two motorized wheels 44A and 44B which have a series of protuberances 46 which cooperate during rotation to periodically pinch and seal the tubular extrudate as it passes between the wheels 44A and 44B.

Alternatively, a retraction means 48 can be employed to periodically retract the inner bore so as to interrupt the flow of the cell suspension. The effect of these retractions is to periodically seal the tubular extrudate and again form multiple compartments. In yet another alternative approach, the controller 38 can vary the pressure applied by pump 24 (and/or pump 28) to create periodic interruptions in the flow of the cell suspension.

In FIG. 2, the extrusion head 14 is shown in more detail, including an inner bore 16 for delivery of a cell suspension and an outer bore 18 for delivery of a polymeric solution. As the cell suspension and the polymeric solution are extruded through the common extrusion pore 19, the polymeric solution coagulates to form an outer coating about the cell suspension.

In FIG. 3, an alternative extrusion head 14A is shown in more detail comprising an inner bore 16 for the delivery of the cell suspension, a second bore 18 (surrounding the inner bore) for delivery of the polymeric solution, and an outer most bore 20 for delivery of a flowing quenchent fluid, such as saline. In this embodiment, a smooth coating can be obtained by simultaneously extruding the cell suspension and polymeric solution through common pore 19 while applying a flowing quenchent fluid during the extrusion (e.g., from the outer most bore 20 in the extrusion head assembly 14A.)

In FIG. 4, the sealing element 42 of FIG. 1 is shown in more detail. Motorized wheels 44A and 44B are mounted on opposite sides of the tubular extrudate 12, such that upon rotation protuberances 46 on the wheels periodically come in contact with the extrudate 12 to pinch and seal the extrudate 12 as it exits the extrusion head 14. The wheels 44A and 44B can be mechanically linked and operated by a conventional motor under the control of a controller, such as shown in FIG. 1. The result of the periodic sealing of the extrudate 12 is a multi-compartment macrocapsule strand 50 having a polymeric membrane 52 surrounding an encapsulated cell solution 54 with individual cells 56 disposed therein. The individual cell capsules are joined to each another by connective filaments 58 where the protuberances 46 of the sealing means 42 has pinched the extrudate 12.

Various polymers can be used to form the membrane coatings of the present invention, including polymers derived from solutions which would otherwise be incompatible with the propagation of living cells. Because of the unique extrusion process disclosed in the present invention, solvents which would otherwise be toxic are quickly driven away from the aqueous cell suspension during the membrane formation process, thereby permitting the use of many new and potentially useful polymeric materials. For example, polymeric membranes can be formed from polyacrylates (including acrylic copolymers), polyvinylidienes, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyacrylonitriles, as well as derivatives, copolymers, and mixtures thereof.

The solvent for the polymer solution will depend upon the particular polymer chosen for the membrane material. Suitable solvents include a wide variety of organic solvents, such as alcohols and ketones, generally, as well as dimethylsulfoxide (DMSO), dimethyacetamide (DMA) and dimethylformimide (DMF), in particular. In general, water-miscible organic solvents are preferred.

The polymeric solution or "dope" can also include various additives, including surfactants to enhance the formation of porous channels, as well as antioxidants to sequester oxides that are formed during the coagulation process. Moreover, when the cell capsules of the present invention are designed for implantation, materials, such as anti-inflammatory agents and cell growth factors, can also be incorporated into the polymeric membrane to reduce immune response or stimulate the cell culture, respectively. Alternatively, these materials can be added to the multi-compartment cell capsule strands after formation by a post-coating or spraying process. For example, the strands can be immersed in a solution which contains an anti-inflammatory agent, such as a corticoid, an angiogenic factor, or a growth factor following extrusion to post-coat the cell capsules.

Post coating procedures can also be used to provide a protective barrier against immunogens and the like. For example, after formation, the cell capsule strands can be coated (e.g., by immersion, spraying or applying a flowing fluid during extrusion) with a surface protecting material, such as polyethylene oxide or polypropylene oxide (e.g., having a molecular weight of about 10,000 Daltons or greater), to inhibit protein interactions with the capsules.

Various techniques can also be employed to control the smoothness or roughness of the outer surface of the polymeric coating. In some instances, a very smooth outer coating can be preferable to reduce scar tissue attachment and other immunoreactions during implantation. Such a smooth coating can be obtained by simultaneously immersing the tubular extrudate in a quenchent, such as a bath of physiological saline, or by applying a flowing, quenchent fluid during the extrusion (e.g., from a third, concentric, outermost bore in an extrusion head assembly). Alternatively, in some applications a rough outer surface with larger pores may be desired, for example, in instances where capillary ingrowth during implantation is desired, and such a rougher outer surface can be obtained by coagulation in air.

Various cell lines can be encapsulated according to the present invention. As noted above, the multi-compartment cell culture strings are particularly useful for the constitutive delivery of neurotransmitters, such as dopamine, which is secreted by cells of the adrenal medulla, embryonic ventral mesencephalic tissue and neuroblastic cell lines. PC12 cells (an immortalized cell line derived from a rat pheocromocytoma) are particularly preferred in some applications because of their ability to secrete large amounts of dopamine over long periods of time. Other neurotransmitters include gamma aminobutyric acid (GABA), serotonin, acetylcholine, noradrenaline and other compounds necessary for normal nerve functions. A number of cell lines are known or can be isolated which secrete these neurotransmitters. Cells can also employed which synthesize and secrete agonists, analogs, derivatives or fragments of neurotransmitters which are active, including, for example, cells which secrete bromocriptine, a dopamine agonist, and cells which secrete L-dopa, a dopamine precursor.

In other embodiments of the invention, the encapsulated cells can be chosen for their secretion of hormones, cytokines, nerve growth factors, angiogenesis factors, antibodies, blood coagulation factors, lymphokines, enzymes, and other therapeutic agents.

The aqueous cell suspensions can further include various additives to protect the cells during the extrusion process or to stimulate their growth subsequently. Such additives can include, for example a nutrient medium or growth factors which are incorporated into the aqueous suspension, as well as an anchorage substrate material to enhance cell attachment. The anchorage substrate material can be a proteinaceous material, such as collagen, laminin, or polyamino acids. Alternatively, the cell suspension or the polymeric solution (or both) can include a foaming agent or a blowing agent which can distort the inner surface of the polymeric coating to increase the anchorage surface area of the tubular interior.

The products of the present invention can take various forms, including simple tubular extrudates as well as multi-compartment cell capsule strings. The shape of the multi-compartment strings can be tubular, resembling sausages, or nearly spherical, resembling strings of pearls. The maximum outer diameter of the strand with typically range from about 0.1 to about 1.0 millimeters. The membrane wall thickness will typically range from about 10 to about 100 micrometers. The strand length of the strands will vary depending upon the particular application.

The products can also take the form of "tethered" cell capsules, that is, one or more individual cell compartments corrected to a long polymeric tube or string. In FIG. 5, such a tethered cell capsule 51 is shown having a polymeric membrane 52 surrounding an encapsulated cell solution 54 with individual cells 56 disposed therein. The cell capsule 51 further includes a long polymeric filament 59 which can be formed by the same apparatus as described above in connection with FIG. 4 by interrupting the flow of the cell solution and constraining the polymeric solution to form a solid tether. The tether also can be post coated with a material (e.g., a polyurethane or the like) which imparts additional strength to the filament. Such tether cell capsules can find a variety of applications, particularly when implanted in a subject for constitutive delivery of active factors. In use, the cell capsule can be located as close to the target region (e.g., in the brain, peritoneal cavity or elsewhere) as desired while the other end of the tether can be fixed at a convenient anchor point or disposal in a readily accessible location for retrieval.

The invention will next be described in connection with certain illustrative, non-limiting examples:

EXAMPLES

An extrusion system similar that illustrated in FIG. 1 was used, consisting of three electronically controlled programmable infusion pumps, a jet spinneret, two motor-controlled, coaxial wheel systems on the perimeter of which occluding polytetrafluoroethylene tubes were mounted, and an uptake system.

The macrocapsules were formed by injection of a polymeric solution into the outer tube of the spinneret. A coagulant, typically the encapsulated cells in their culture medium, was simultaneously injected in the spinneret inner tube. The encapsulating membrane was formed by a dry-jet, wet spinning process, i.e., the fast stabilization of the polymer solution emerging from the spinneret nozzle by the internal quench medium coupled with further stabilization in a quench bath. The closure of the fiber was performed by mechanically squeezing the forming hollow fiber with the coaxial wheel system prior to immersion in the quench bath. Near the spinneret head, the solvent concentration was sufficiently high to allow proper fusion of the fiber wall. Following each round of encapsulation, pure solvent was flushed automatically through the lumen of the spinneret to avoid clogging of the nozzle.

PC12 cells, an immortalized cell line derived from a rat pheocromocytoma which secretes large amounts of dopamine, were cultivated on collagen-coated tissue culture dishes in RPMI 1640 medium supplemented with 10% heat inactivated horse serum and 5% fetal calf serum. Dissociated bovine adrenal medullary cells, a non-dividing cell type which secretes dopamine, were maintained in DMEM medium supplemented with 5% fetal calf serum. Prior to encapsulation, the cells were harvested and loaded at a concentration of 1 X 10^5 cells/ml in a 3 ml syringe. A 15 percent vinylchloride-acrylonitrile copolymer solution in either dimethylsulfoxide (DMSO), dimethylformimide (DMF), or dimethylacetamide (DMAC) was loaded into a 5 ml glass syringe. Both solutions were then coextruded through the spinneret, and the capsules were collected in a physiologic saline solution. The capsules were rinsed and placed in individual wells containing the appropriate culture media.

Basal and potassium-evoked release of catecholamines was quantified under static incubation conditions by ion-pair reverse-phase high performance liquid chromatography (HPLC) equipped with electrochemical detection at 2 and 4 weeks. Morphological analysis, including light, scanning, and transmission electron microscopy, was performed on representative samples for each time period.

All cell-loaded capsules released dopamine into the medium under basal conditions at all time periods. High potassium treatment increased dopamine release from both PC12 and adrenal medullary cells. Dopamine output by PC12 cells, but not adrenal medullary cells, increased with time. The increase in dopamine release by the PC12 cell-loaded capsules over time is believed to be related to cell proliferation within the polymer capsule. No significant difference in dopamine release could be observed from PC12-loaded capsules extruded with the three different solvent systems (DMSO, DMF, DMAC), which suggests that the encapsulation technique of the present invention may prevent cell damage inflicted by solvents (FIG. 6). Due to the higher pressure of the inner bore system, the solvent was quickly driven toward the outside of the polymer capsule which prevented extended cell-solvent contact.

Morphological analysis revealed the presence of small clusters of PC12 cells randomly dispersed throughout the lumen of the capsule. At the electron microscope level, well-preserved PC12 cells, with their typical electron-dense secretory granules, could be observed. Cell division within the capsule space was suggested by the presence of numerous mitotic figures. Although initially coextruded as a cell suspension, adrenal chromaffin cells formed packed aggregates one week after encapsulation.

FIG. 7 shows the results of an in vitro assay in which PC12 cells were encapsulated according to the present invention and monitored for release dopamine at two and four weeks following encapsulation. Dopamine levels were measured under both normal (controlled) conditions and also under a high potassium stimulation, which is known to induce depolarization of the cells and, consequently, to increase the secretion of dopamine in viable cells. As can be seen from the graph, there was little activity at two weeks; however, at four weeks the encapsulated cells exhibited dopamine secretions not only under normal conditions but also exhibited a strong response to the potassium stimulation, indicating that the cells were indeed viable in their encapsulated state.

FIG. 8A and 8B shows the results of further in vitro assays in which the secretions of PC12 cells and chromaffin cells, respectively, were monitored four weeks after encapsulation according to the present invention. Again, the cells were stimulated by high potassium concentrations and the medium while the PC12 cells released only dopamine, the chromaffin cells released a variety of catecholamines. The graft shows the levels of noradrenaline (NE), epinephrine (EPI), and dopamine (DA).

Due to their fluid dynamics, the macrocapsules extruded in accordance with the present invention will allow the use of a wider range of polymer/solvent systems and can constitute a more efficient encapsulation technique. The results show that immortalized and differentiated dopamine-secreting cells will survive in macroencapsulation. The ability of these capsules to spontaneously release dopamine over time suggests that polymer encapsulation can provide an alternative to the transplantation of non-encapsulated or microencapsulated dopamine-secreting cells in the treatment of Parkinson's disease.

Claims

1. A method of encapsulating viable cells, the method comprising co-extruding an aqueous cell suspension and a polymeric solution through a common extrusion port to form a tubular extrudate having a polymeric outer coating which encapsulates said cell suspension.

2. The method of claim 1 wherein the method further comprises extruding the aqueous cell suspension and the polymeric solution through a common extrusion port having at least two concentric bores, such that said cell suspension is extruded through an inner bore and said polymeric solution is extruded through an outer bore.

3. The method of claim 1 wherein the method further includes sealing the ends of said tubular extrudate.

4. The method of claim 1 wherein the method further includes sealing the tubular extrudate at intervals to define separate cell compartments connected by polymeric links.

5. The method of claim 4 wherein the step of sealing the extrudate further comprises compressing the extrudate at intervals to define separate cell compartments.

6. The method of claim 4 wherein the step of sealing the extrudate further comprises modifying the pressure under which the cell suspension or the polymeric solution is extruded, thereby collapsing the tubular extrudate at intervals to define separate cell compartments.

7. The method of claim 4 wherein the step of sealing the extrudate further comprises impeding the flow of the cell suspension at intervals to collapse the tubular extrudate and define separate cell compartments.

8. The method of claim 1 wherein the polymeric coating is coagulated in air.

9. The method of claim 1 wherein the polymeric coating is coagulated in a reduced pressure chamber.

10. The method of claim 1 wherein the polymeric coating is coagulated in a quenchent bath.

11. The method of claim 1 wherein the method further comprises extruding an aqueous suspension containing cells that secrete a biologically-active factor.

12. The method of claim 1 where in the method further comprises extruding an aqueous suspension containing cells that secrete a neurotransmitter.

13. The method of claim 1 wherein the method further comprises extruding an aqueous cell suspension which contains a nutrient medium.

14. The method of claim 1 wherein the method further comprises extruding an aqueous cell suspension which also contains an anchorage substrate.

15. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises a water-miscible solvent component.

16. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises a surfactant.

17. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises an anti-inflamatory agent.

18. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises an antioxidant.

19. The method of claim 1 wherein the method further comprises controlling the viscosity of said polymeric solution, such that upon coagulation said outer polymeric coating will form a semipermeable membrane about said cell suspension.

20. The method of claim 1 wherein the method further comprises maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion to impede solvent diffusion from said polymeric solution into said cell suspension.

21. The method of claim 1 wherein the method further comprises applying a protective barrier material to the outside of the polymeric coating.

22. A tubular extrudate prepared by the method of claim 1.

23. A cell culturing vehicle comprising a tubular, semipermeable, polymeric membrane encasing a cell culture, said tubular membrane being formed by extrusion.

24. The vehicle of claim 23 wherein the membrane is permeable to molecules having a molecular weight of about 150,000 or less.

25. The vehicle of claim 23 wherein the maximum outer diameter of the cell culture vehicle ranges from about 0.1 to about 1.0 millimeters.

26. The vehicle of claim 23 wherein the membrane has a wall thickness ranging from about 10 to about 100 microns.

27. The vehicle of claim 23 wherein the polymeric membrane further comprises a polyacrylate material.

28. The vehicle of claim 23 wherein the polymeric membrane further comprises a surfactant.

29. The vehicle of claim 23 wherein the polymeric membrane further comprises an anti-inflamatory agent.

30. The vehicle of claim 23 wherein the polymeric membrane further comprises an anti-oxidant.

31. The vehicle of claim 23 wherein the polymeric solution further comprises a water-miscible solvent.

32. The vehicle of claim 23 wherein the extruded tubular membrane is sealed at intervals to define separate cell compartments connected by polymeric links.

33. The vehicle of claim 23 wherein the extruded tubular membrane is sealed to define at least one cell compartment connected to a tethering filament.

34. The vehicle of claim 23 wherein the cell culture further comprises an aqueous cell suspension containing cells that secrete a biologically-active factor.

35. The vehicle of claim 34 wherein the aqueous cell suspension further comprises a nutrient medium.

36. The vehicle of claim 23 wherein the cell culture further comprises an anchorage substrate material.

37. The vehicle of claim 36 wherein the anchorage material comprises a collagen material.

38. The vehicle of claim 36 wherein the anchorage material comprises a laminin material.

39. The vehicle of claim 36 wherein the anchorage material comprises a polyamino acid.

40. The vehicle of claim 23 wherein the vehicle further comprises a protective barrier material which coats at least a portion of the outer surface of the polymeric membrane.

41. The vehicle of claim 40 wherein the protective barrier material is an inhibitor of protein interactions.

42. The vehicle of claim 40 wherein the protective barrier material is selected from the group consisting of polyethylene oxides, polypropylene oxides, derivatives and mixtures thereof.

43. The vehicle of claim 23 wherein the cell culture further comprises a culture of cells capable of secreting a therapeutic factor.

44. A system for encapsulating viable cells, the system comprising:

an extrusion head assembly having at least a first inner bore and a second, concentric, outer bore;

cell suspension supply means for supplying an aqueous cell suspension to the inner bore of said extrusion head assembly; and

polymeric solution supply means for supplying a polymeric solution to the outer bore of the extrusion head assembly, such that said cell suspension and said polymeric solution can be co-extruded to form a tubular extrudate having a polymeric outer coating which encapsulates said cell suspension.

45. The system of claim 44 wherein the system further comprises flow control means for maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion.

46 The system of claim 44 wherein the flow control means comprises a computer adapted to control a first infusion pump in said cell suspension supply means and a second infusion pump in said polymeric solution supply means.

47. The system of claim 44 wherein the system further comprises a quenchent bath for coagulating said polymeric solution following extrusion.

48. The system of claim 44 wherein the system further comprises sealing means for sealing the tubular extrudate at intervals to define separate cell compartments connected by polymeric links.

49. The system of claim 48 wherein the sealing means further comprises means for compressing the extrudate at intervals during extrusion to define separate cell compartments.

50. The system of claim 44 wherein the sealing means further comprises means for modifying the pressure under which the cell suspension or the polymeric solution is extruded, thereby collapsing the tubular extrudate at intervals to define separate compartments.

51. The system of claim 44 wherein the sealing means further comprises means for impeding the flow of the cell suspension at intervals to collapse the tubular extrudate and define separate cell compartments.

52. The system of claim 44 wherein the sealing means further comprises a retraction means for moving the inner bore of said extrusion head assembly relative to the outer bore to interrupt the flow of the tubular extrudate at intervals to define separate cell compartments.

53. The system of claim 44 wherein the extrusion head assembly includes a third, concentric, outer most bore for delivery of a quenchent fluid to coagulate said polymeric solution.

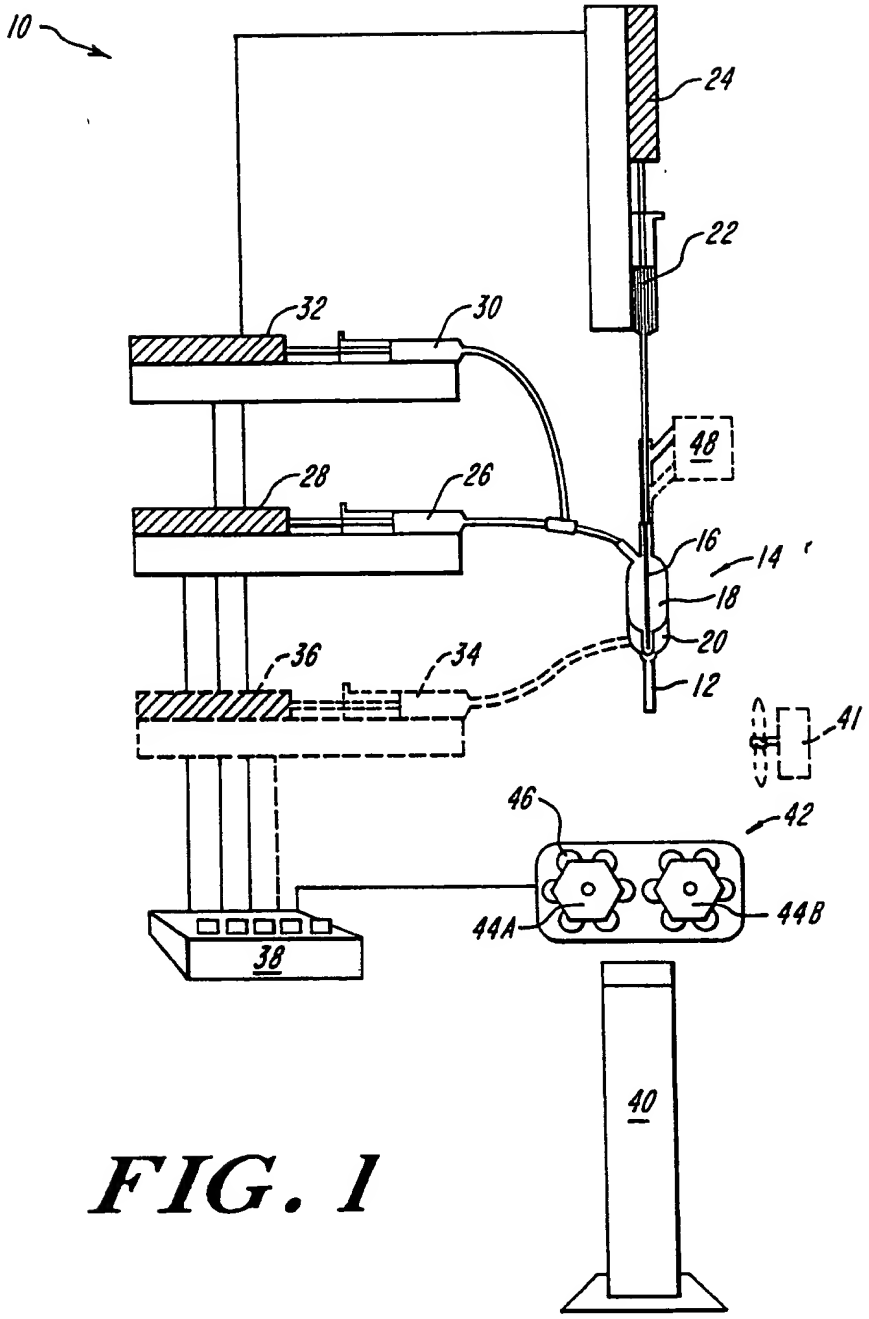


FIG. 1

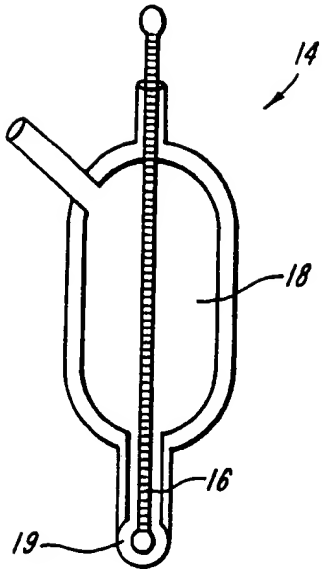


FIG. 2

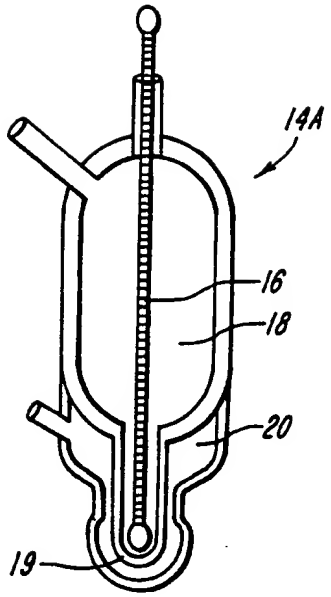


FIG. 3

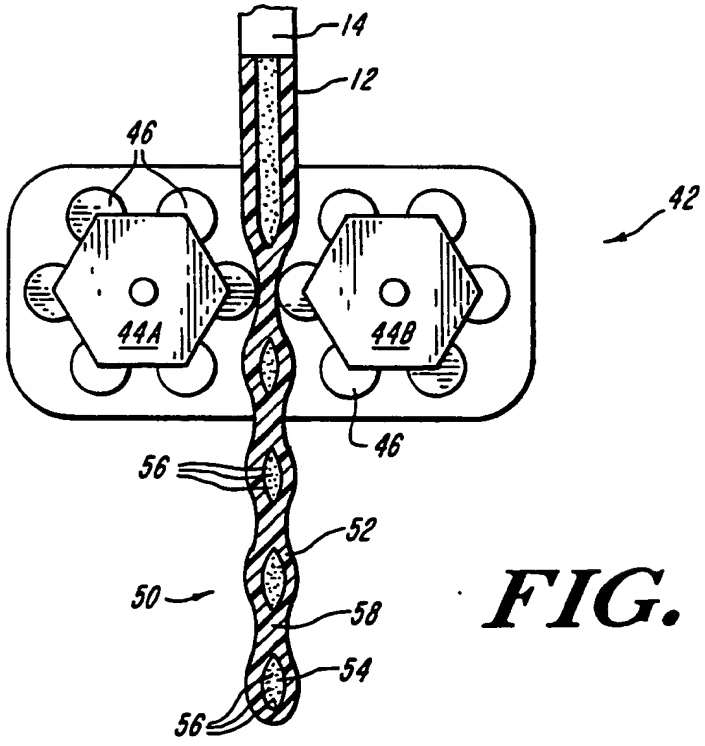
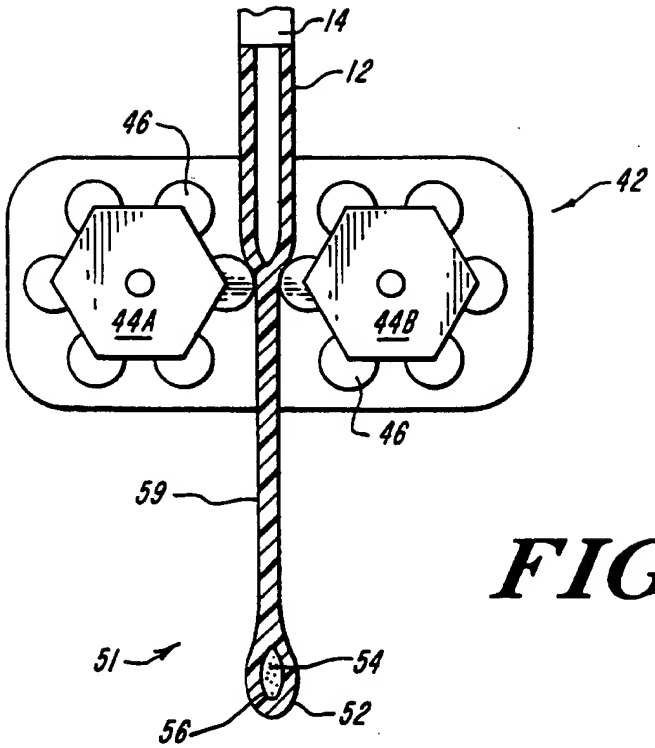


FIG. 4


14
12
46
42
44A
44B
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52
56

FIG. 5

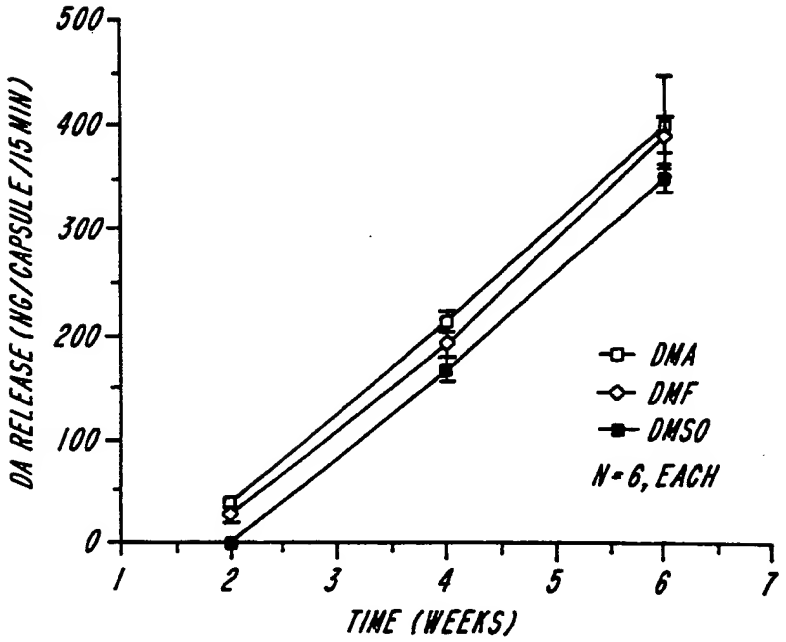
500
400
300
200
100
0
DA RELEASE (NG/CAPSULE/15 MIN)
1
2
3
4
5
6
7
TIME (WEEKS)
DMA
DMF
DMSO
N=6, EACH

FIG. 6

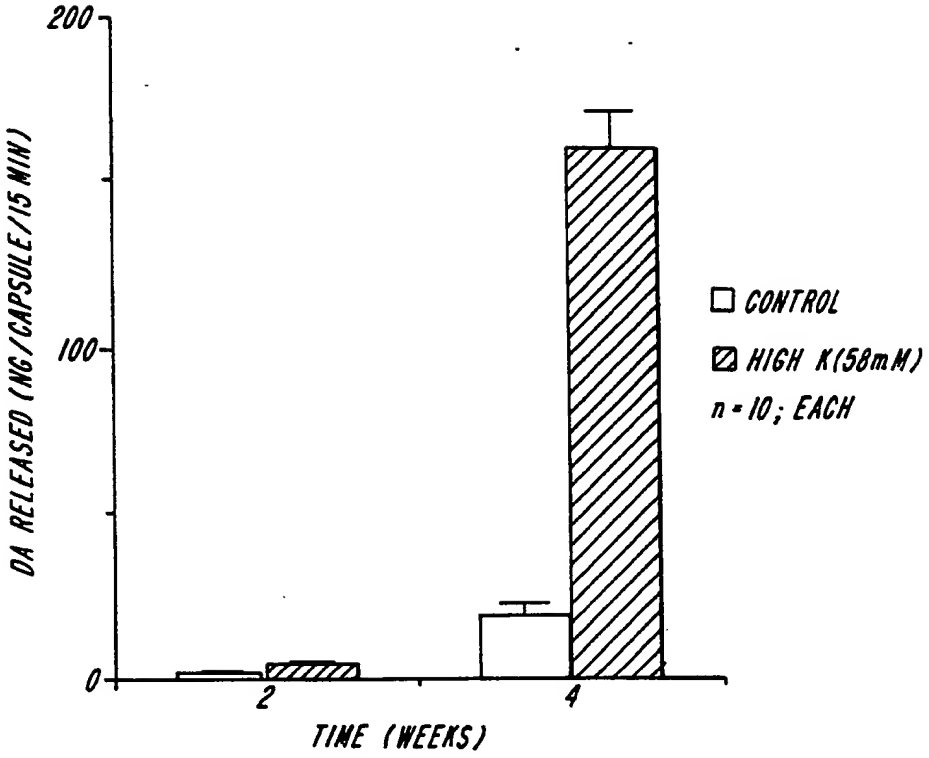

FIG. 7

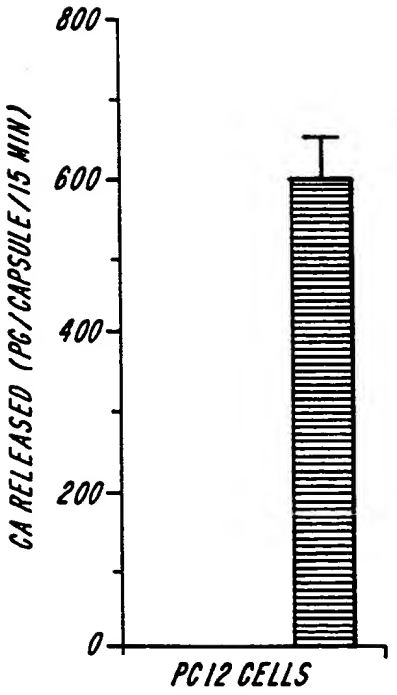

FIG. 8A

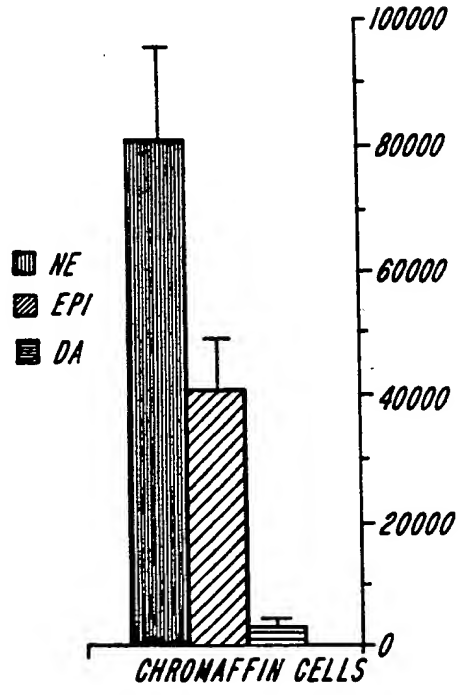

FIG. 8B

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/00157

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 9/50, C 12 N 11/04, B 01 J 13/04

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K, B 01 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	FR, A, 2599639 (RAMOT UNIVERSITY AUTHORITY) 11 December 1987 see page 3, lines 18-33; page 4, lines 27-35; page 5, lines 29-35 --	1-4,8,10, 14,21-23,25, 34,36,44,45, 53
X	Patent Abstracts of Japan, vol. 11, no. 315 (C-451)(2762), 14 October 1987 & JP, A, 62100288 (HITACHI PLANT ENG & CONSTR. CO. LTD) 9 May 1987 see the abstract --	1
A	FR, A, 2336176 (MORISHITA JINTAN CO.) 22 July 1977 see page 1, lines 1-6; page 2, lines 27-39; page 5, lines 25-37 --	1-5
A	EP, A, 0188309 (CONNAUGHT LABORATORIES) 23 July 1986 ./.	1,25,27,34, 40

* Special categories of cited documents: [10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18th April 1991	07. 06. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Danielle van der Haas

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	see page 1, lines 19-22; page 3, lines 20-27	
	--	
A	WO, A, 8704367 (LTL ASSOCIATES) 30 July 1987 see page 5, lines 21-32; page 7, lines 4-12; page 9, lines 3-32; page 12, lines 14-27	1,3,4,27,34, 43
	--	
A	US, A, 4892538 (P.AEBISCHER et al.) 9 January 1990 see column 8, lines 18-46 (cited in the application)	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9100157
SA 44283

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 04/06/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP-A- 63146792	18-06-88
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		JP-A- 52148663	10-12-77
		JP-C- 924406	22-09-78
		JP-A- 52078775	02-07-77
		JP-B- 53001067	14-01-78
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		EP-A- 0388428	26-09-90
		WO-A- 8904655	01-06-89

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(30) Priority data:
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(74) Agents: ENGELLENNER, Thomas, J. et al.; Lahive & Cockfield, 60 State Street, Suite 510, Boston, MA 02109 (US).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent).

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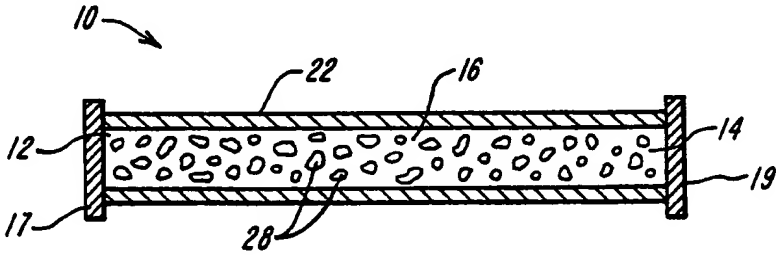
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DEVICES AND METHODS FOR ENHANCED DELIVERY OF ACTIVE FACTORS



(57) Abstract

Methods and devices (10, 20, 30) are disclosed for the enhanced delivery of an active factor from an implanted, active factor-secreting cell culture to a target region in a subject. The cell culture (25) is maintained within a biocompatible, semipermeable electrically charged membrane (22) which generates electric charges on its surface, thereby resulting in enhanced delivery of active factor therefrom. The membrane permits the diffusion of active factor and metabolites therethrough, while excluding viruses, antibodies, and other detrimental agents present in the external environment from gaining access. In addition, implantable cell culture devices are disclosed which may be retrieved from the subject, replaced, or recharged with new, active factor-secreting cell cultures, and reimplanted.

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DEVICES AND METHODS FOR ENHANCED DELIVERY OF ACTIVE FACTORS

Background of the Invention

The technical field of this invention is the treatment of deficiency diseases and, in particular, the treatment of diseases or disorders resulting from an inadequacy or lack of a neurotransmitter, growth factor, or hormone.

Neurotransmitters, growth factors, and hormones are soluble, "trans-acting" molecules that are elicited by one cell and affect another cell. For example, neurotransmitters act as chemical means of communication between neurons. They are synthesized by the presynaptic neuron and released into the synaptic space where they are then taken up by postsynaptic neurons. Lack of neurotransmitter-mediated synaptic contact causes neuropathological symptoms, and can also lead to the ultimate destruction of the neurons involved. In fact, neurotransmitter deficits have been implicated in a number of neurological diseases.

It has been discovered that localized delivery of the relevant neurotransmitter to the target tissue may reverse the symptoms without the need for specific synaptic contact. For example, paralysis agitans, more commonly known as Parkinson's disease, is characterized by a lack of the neurotransmitter, dopamine, within the striatum of the brain, secondary to the destruction of the dopamine secreting cells of the substantia nigra. Affected subjects demonstrate a stooped posture, stiffness and slowness of movement, and rhythmic tremor of limbs, with dementia being often encountered in very advanced stages of the disease. These clinical symptoms can be improved by the systemic administration of dopamine precursors, such as L-dopa (Calne et al., (1969) Lancet ii:973-976), which are able to cross the blood-brain barrier and there to be converted into dopamine, or agonists such as bromocriptine (Calne et al., (1974) Bri. Med. J. 4:442-444) which elicit a dopamine response. Dopamine, itself, cannot be administered systemically because of its inability to cross the blood-brain barrier.

One of the drawbacks of direct systemic therapy is that other neighboring organs or tissues that respond to the administered compound are also affected. In addition, it may become increasingly difficult to administer the correct dosage of compound with time because the "therapeutic window" narrows. For example, just after L-dopa administration, the patient is overdosed, exhibiting excessive spontaneous movement; some time thereafter

the drug level may become insufficient, causing the patient to again express Parkinsonian symptoms.

Therefore, what is needed is a method of delivering an appropriate concentration of a needed compound or "active factor" to a target region of the body which is deficient in that factor.

The constitutive provision of the needed quantity of active factor has been proposed to alleviate the aforementioned problems. To this end, the transplantation of neurotransmitter- or growth factor-secreting cells has been attempted. For example, dopamine-secreting tissue has been transplanted into the brain to treat Parkinsonian symptoms. However, recent studies have shown that although the brain is considered "immuno-priviledged", rejection ultimately occurs with both allografts (identical tissue from another of the same species) and xenografts (similar tissue from another of a different species). The rejection problem necessitates the co-adminstration of immuno-suppressors, the use of which renders their own set of complications and deleterious side-effects.

To prevent the elicitation of an immune response, remedial transplantation of neuro-transmitter-secreting tissue has been tried using the patient's own tissue. For example, dopamine-secreting tissue from the adrenal medulla of patients suffering from Parkinson's disease has been implanted in their striatum with reasonable success. However, this procedure is only used in patients less than 60 years of age, as the adrenal gland of older patients

may not contain sufficient dopamine-secreting cells. This restriction limits the usefulness of this procedure as a remedy since the disease often affects older people.

An alternative way of alleviating an immune reaction in response to tissue transplantation involves protecting the cells to be implanted in a selectively permeable membrane. Such a membrane allows the diffusion therethrough of metabolites and active factors, while preventing the passage of antibodies and complement as well as viruses. However, in some systems, cell-to-cell contact is thought to be a prerequisite for the elicitation of active factor in the concentration normally required by a target tissue. In addition, diffusion rates through the membrane may change the delivery rate of an active factor from the cell culture to the target tissue.

Therefore, there exists a need for improved therapies for various deficiency diseases in general, and in particular, a need for systems which can augment or replace dysfunctional areas of an active factor-producing tissue. More specifically, there exists a need for a method of providing an active factor to a localized area of the body of a subject, the correct dosage of which will be constitutively delivered over time.

Accordingly, it is an object of the present invention to provide a method for delivering an active factor to a localized region of a subject. It is another object of the present invention to provide

a method of enhancing the delivery of active factor from encapsulated cells. Another object is to provide a method of constitutively delivering the needed dosage of an active factor to a subject deficient in that factor.

Another object is to provide an implantable cell culture device which allows for the enhanced delivery of an active factor from the cells cultured within. Yet another object is to provide a cell culture device which is retrievable, and whose contents are renewable with new and/or additional neurotransmitter-secreting cells. A further object is to provide a cell culture device which protects the cells therein from an immunological response or from viral infection, while allowing the delivery of an active factor and metabolites therefrom.

Summary of the Invention

Methods and devices are disclosed herein for the constitutive and enhanced delivery of an active factor to a subject suffering from a deficiency or dysfunction. The term "active factor" is used herein to describe neurotransmitters, growth factors, peptide hormones, trophic factors, lymphokines, and any molecule synthesized and secreted by a cell and required for the proper function and maintenance of a tissue.

It has been discovered that the delivery of an active factor from active factor-secreting cells can be greatly enhanced by encapsulating the cells in a semipermeable, electrically-charged or conductive membrane. This membrane produces surface electric charges that depolarize the encapsulated cells therein, thereby enhancing the delivery of active factor therefrom.

The term "electret" as used herein is intended to encompass natural and synthetic materials capable of generating electrical charges on their surface. Piezoelectric materials are one type of electret which generates a transient electric surface charge upon mechanical stress or deformation. Medical devices employing such electret membranes are disclosed for use in implantable cell culture devices.

The membranes of the present invention are also "semipermeable", or selectively permeable to nutrients, metabolites, and active factors having

molecular weights of about 50,000 daltons or less; excluded from passage are cells, antibodies, complement, virus and other agents harmful to the cells encapsulated therein.

In one preferred embodiment of the invention, the electret membrane is a tube composed of a piezoelectric material such as polyvinylidene difluoride (PVDF), trifluoroethylene (TrFE), or a copolymer thereof (PVDF-TrFE). Alternatively, the membrane can comprise a permanent poled polymeric material or may be fabricated in whole or in part of a conductive polymer.

In accordance with the method of present invention, at least one active factor-secreting cell, such as a neuron, is encapsulated within such a membrane and implanted into a subject where it is maintained protectively while delivering active factor to the local internal environment of that subject.

The cells to be implanted may be any cells which synthesize and secrete a desired active factor. Preferred active factors include neurotransmitters, growth factors, and active analogs, fragments, and derivatives thereof. One particularly useful active factor is the neurotransmitter, dopamine, which is secreted by cells of the adrenal medulla, embryonic ventral mesencephalic tissue, and neuroblastic cell lines such as PC12, a cell line derived from a rat pheochromacytoma. Other useful active factors include the dopamine precursor, L-dopa, and the

dopamine agonist, bromocriptine. A preferred growth factor is fibroblast growth factor (FGF) in either its acidic or basic form.

In one aspect of the invention the encapsulating membrane is a tube preferably having a diameter of about 200 - 600 mm, and a wall thickness ranging from about 50 - 100 mm. The openings of the tube may be covered by removable plugs or caps. Such a construct enables the easy replacement of cells within the membrane with other cells through the uncovered tube openings after retrieval from the subject via the attached guide wire.

Also disclosed is a method for enhancing the secretion of an active factor from an active-factor secreting cell. The method includes the steps of encapsulating the active factor-secreting cell in a semipermeable, electret membrane, and allowing the membrane to generate an electric surface charge, thereby causing the encapsulated cells to become depolarized and to secrete active factor. In a preferred embodiment, the cells is encapsulated within a piezoelectric membrane which generates the depolarizing surface charge upon mechanical stress.

Further a method for providing an active factor to a target region of the body is disclosed. In this method, an active factor-secreting cell is encapsulated within a semipermeable, electret membrane that is permeable to the active factor. The encapsulated cell is implanted in a target region of the body. Enhanced secretion of the active factor from the encapsulated cell results when the

encapsulating membrane generates a surface charge and depolarizes the cells therein.

The invention will next be described in connection with certain illustrated embodiments. However, it should be clear that various modifications, additions, and subtractions can be made without departing from the spirit or scope of the invention. The present invention should not be read to require, or be limited to, particular cell lines or electret materials described by way of sample or illustration. Additionally, although the culture devices described below are generally tubular in shape, it should be clear that various alternative shapes can be employed as well. Moreover, the electrically charged or conductive materials employed in the present invention need not form the entire encapsulating membrane but rather can be interspersed within an otherwise inactive membrane matrix.

Brief Description of the Drawings

The invention itself can be more fully understood from the following description when read together with the accompanying drawings in which:

FIG. 1 is a schematic illustration of an implantable cell culture device for delivering a active factor, according to one aspect of the invention;

FIG. 2 is a schematic illustration of an implantable and retrievable cell culture device for delivering an active factor, according to another aspect of the invention; and

FIG. 3 is a schematic illustration of an implantable, retrievable, and rechargeable cell culture device for delivering an active factor, according to yet another aspect of the invention.

Detailed Description

Methods and devices are disclosed herein for the enhanced delivery of an active factor from an implanted, active factor-secreting cell culture to a target region in a subject. This invention exploits the discovery that upon depolarization, nerve cells may be induced to release neurotransmitter from secretory granules in their cytoplasm.

In the present invention, a cell culture is maintained within a biocompatible, semipermeable electret membrane. The surface charges generated by the membrane causes the encapsulated cells therein to become depolarized and to secrete active factor. The electret membrane is also semipermeable, permitting the diffusion of active factor and metabolites therethrough, while excluding viruses, antibodies, and other detrimental agents present in the external environment from gaining access. In addition, implantable cell culture devices are disclosed which may be retrieved from the subject, replaced, or recharged with new, active factor-secreting cell cultures, and reimplanted.

Any cell line or tissue which secretes a needed active factor can be encapsulated for use in the present invention. These include tissue fragments and established cell lines that secrete active factors *in vivo* such as adrenal medulla, embryonic ventral mesencephalic tissue, neuroblastic cell lines, and PC12 cells, all of which secrete dopamine. Other useful neurotransmitters include gamma aminobutyric acid (GABA), serotonin,

acetylcholine, noradrenaline, and other compounds necessary for normal nerve functions. Preferred active factors is fibroblast growth factor in either its acidic or basic form and various peptide hormones.

In addition, any cell which secretes a precursor, analog, derivative, agonist or fragment of a desired active factor having similar activity can be used, including, for example, cells which elicit L-dopa, a precursor of dopamine, or bromocriptine, a dopamine agonist.

The cells to be encapsulated may be allografts, or cells from another of the same species as the subject in which they are to be implanted, or they may be xenografts, or those from another of a different species. They may be derived from, or are a component of an adult body organ or tissue which normally secretes a particular active factor in vivo. Alternatively, useful cells include embryonic active factor-secreting cells from, for example, the embryonic ventral mesencephalon, neuroblastoid cell lines, or the adrenal medulla.

Further, any cells which have been genetically engineered to express an active factor or its agonist, precursor, derivative, analog, or fragment thereof which has similar neurotransmitter activity are also useful in practicing this invention. Thus, in such an approach, the gene which encodes the active factor, or an analog, precursor, or fragment thereof, is either isolated from a cell line or constructed by various known engineering methods. The gene is then incorporated into a

vector, which, in turn, is transfected into a host cell for expression (see, e.g., Maniatis et al., Molecular Cloning (1982), herein incorporated by reference for further discussion of cloning vehicles and gene manipulation procedures). Appropriately transformed cells which express the active factor can be cultured in vitro until a suitable density is achieved.

The active factor-secreting cells are then placed into the cell culture device prior to implantation, either as tissue fragments or as seed cultures.

The device is a membrane adapted to receive the active-factor secreting cells. This membrane is biocompatible and semipermeable so as to protect the cells from deleterious encounters with viruses and elements of the immune system. Such protection is particularly important for preserving allografts or xenografts which are eventually considered foreign even in the "immuno-priviledged" brain. Therefore, the membrane should bar viruses, macrophages, complement, lymphocytes, and antibodies from entry while allowing the passage of nutrients, gases, metabolic breakdown products, other solutes, and the neurotransmitter to pass therethrough. Accordingly, a biocompatible and nonresorbable materials having pores enabling the diffusion therefrom of molecules having a molecular weight of up to about 50,000 daltons are useful for practicing the present invention.

Further, the membrane of the cell culture device is made of an electret material, one preferred example of which is a piezoelectric material. Useful piezoelectric membranes are composed of biocompatible, semicrystalline polymers which may have to be poled prior to use, but which need not be stretched prior to poling. Poling can then be performed to align the polymeric chain segments in a particular orientation, thereby establishing a predefined dipole moment. Polarization can be achieved, for example, by disposing one electrode on the inside of a tubular membrane and another electrode on the outside of the tube, and then applying a voltage to one of the electrodes such that an electric field is established. When the membrane is a piezoelectric material, poling preferably establishs a charge generation (polarization constant) ranging from about 0.5 - 35 picoColoumbs per Newton, and, more preferably, from about 1.0 - 20.0 picoColoumbs per Newton.

Piezoelectric materials useful in the present invention include a variety of halogenated polymers, copolymers and polymer blends. The halogenated polymers include polyvinylidene difluoride, polyvinyl fluoride, polyvinyl chloride and derivatives thereof as well as copolymers such as copolymers of the above materials and trifluoroethylene. PVDF-TrFE, for example, is a preferable material for membrane fabrication with adequate mechanical characteristics for cell filling and stereotaxic brain implantation. It need not be stretched prior to poling.

Small tubular membranes of PVDF-TrFE can be constructed by various fabrication techniques known

to those skilled in the art, including, for example, the spray-phase inversion technique. In this procedure, the tubular membrane is fabricated using a machine consisting of a small precision lathe in which mandrels of different diameters are rotated using an electronically controlled variable speed motor. A carriage is positioned adjacent to the lathe bed. The carriage can move bidirectionally and in parallel with the rotating mandrel. The carriage is driven by an electronically controlled motor which is automatically reversed by the action of electro-mechanical relays controlled by micro-switches. Two spray-guns are mounted on the carriage and can be fixed at different angles to one another, and at different distances between nozzles and mandrel, so that the jet-streams can be directed on a precise point over the mandrel.

The porosity of the membrane may be controlled by the degree of phase inversion. The advantage of PVDF-TrFE resides in its ability to be electrically poled without the need for mechanical stretching.

The cell culture device may take any shape that will accommodate the cells to be encapsulated, and which will not cause undue trauma upon surgical implantation. In addition, to ensure viability of the cells within, only small diffusion distances are established between the implanted tissue and the vascularized surrounding host tissue. To this end, the diameter of the tube should be in the range of about 200 - 600 m, as determined by the observation

that oxygen tension, when relying only on diffusion, approaches zero in a vascularized tissue.

A preferable implantable cell culture device 10 shown in FIG. 1 is a tubular, selectively permeable piezoelectric membrane 22 having ends 12 and 14 through which active factor-secreting cells 25 are loaded into cell compartment 16. Ends 12 and 14 may then be permanently occluded with caps 17 and 19 or, alternatively, with an epoxy glue or sutures of a biocompatible and nonresorbable material like polypropylene.

The device 10 as shown in FIG. 1 can be surgically implanted into the target region of a subject such that membrane 22 is in immediate contact with body tissues or fluids. The targeted region may be the in vivo site of deficiency, need, or the site of synthesis of the factor. For example, this region may be any part of the nervous system, but will most often be the brain, as it is the source of numerous neurological dysfunctions. The site will provide means for the mechanical deformation of the piezoelectric membrane, necessary for generation of the depolarizing charge. Such means includes the pulsation of adjacent blood vessels and natural movements of the body to which the membrane may be attached.

The method of the present invention may include an additional step whereby the initially encapsulated and implanted cells are retrieved from the subject in the event that they cease to produce active factor, expire, or are no longer needed to

correct the dysfunction. As illustrated in FIG. 2, retrieval of implanted cell culture device 20 can be accomplished by means of guide wire 18 which is permanently attached to end cap 17 or 19. This wire may be constructed of any nonresorbable, biocompatible material with enough tensile strength to support the cell culture device.

A exemplary cell culture device useful in practicing this method is shown in FIG. 3. Device 30 is tubular, having ends 12 and 14 reversibly covered with removable, friction-fitted caps 22 and 24, respectively, to enable the extraction and replacement of cells 25 in cell compartment 16 with new cells. The device 30 as shown in FIG. 3 can be surgically implanted into the brain of a subject such that guide wire 18 is located directly under the epithelial tissues of the head, and membrane 22 is in immediate contact with brain tissue. Transient charges will be generated by the piezoelectric membrane in response to normal movements of the head and brain.

The invention will next be described in connection with the following non-limiting examples.

EXAMPLES

Piezoelectric semipermeable tubular membranes were fabricated on a rotating polyethylene mandrel of 500 mm diameter using a spraying-phase inversion technique. PVDF-TrFE copolymer (70:30 v/v) was obtained from Autochem, NJ. The fabrication process was undertaken in a chemical hood. A skin

was formed by spraying a PVDF-TrFE solution (1% in methyl ethyl ketone (MEK)) or cospraying a 0.5% solution of PVDF-TrFE and a 50% ethanol/water mixture onto the mandrel. Two spray-guns were operated at the same volumetric flux using compressed nitrogen as the transport gas. The spray-guns were positioned at 30°C to one another and aligned so that the jet-streams converged at a distance of 4 cm between the spray-gun nozzles and the rotating mandrel. The mandrel rotation speed and carriage movement speed were fixed at 600 rpm and 70 cm/min, respectively. Upon the completion of 16 passages, a nonsolvent solution of 1:1 H_2O/methanol was simultaneously but separately sprayed by the second spray-gun. The nonsolvent solution allowed the formulation of an outer porous membrane structure which functions as the inner skin structural support.

Following the fabrication process, the polyethylene mandrel was removed and immediately submerged in a bath of 1:1 H_2O/methanol. This step was performed to stabilize the delicate sponge-like structure of the tubular membranes by allowing the solvents to gradually leave and the nonsolvent to enter the porous structure. The tubular membranes were mechanically detached from the mandrel and dried at room temperature.

Cross-section scanning electron micrographs of the tubular membranes showed a thin inner skin surrounded by a porous network.

Tubular membrane segments (1 cm) were cut and one of their extremities capped with a fast

curing acrylic polymer glue. The tubular membranes were either left empty or filled with PC12 cells. The second extremity of the tubes was then capped with the same acrylic polymer glue.

Empty PVDF-TrFE cell culture devices implanted stereotaxically in the striatum of rats, the target structure for dopamine in Parkinson's disease, displayed a mild tissue reaction consisting primarily of microglia and reactive astrocytes.

With the cell-filled devices, intact PC12 cells were observed after 7 days in vitro. TEM micrographs showed the presence of well preserved dopamine-containing secretory granules. Intact viability of macroencapsulated PC12 cells and limited host tissue reaction to the membranes confirmed the utility of PVDF-TrFE as an encapsulation material.

We claim:

1. A medical device for use in providing an active factor to a target region of the body, said device comprising:

a semipermeable, electrically charged membrane; and

at least one active factor-secreting cell encapsulated within said membrane,

said membrane being permeable to said active factor, and impermeable to cells, virus, complement, antibodies and other factors detrimental to said cells.

2. The device of claim 1 wherein said semipermeable membrane is permeable to molecules having a molecular weight of about 50,000 daltons or less.

3. The device of claim 1 wherein said membrane comprises a tube.

4. The device of claim 3 wherein said tube has a diameter of about 200 - 600 mm.

5. The device of claim 1 wherein said membrane has a wall thickness ranging from about 50 - 100 mm.

6. The device of claim 1 wherein said membrane comprises an electret material.

7. The device of claim 1 wherein said membrane comprises a piezoelectric material.

8. The device of claim 1 wherein said membrane comprises polyvinylidene difluoride.

9. The device of claim 1 wherein said membrane comprises trifluoroethylene.

10. The device of claim 1 wherein said membrane comprises a polyvinylidene difluoride-trifluroro-ethylene copolymer.

11. The device of claim 1 wherein said membrane comprises a conductive polymer.

12. The device of claim 1 wherein said active factor-secreting cell is a neuron.

13. The device of claim 1 wherein said active factor is selected from the group consisting of neurotransmitters and precursors, active fragments, analogs, and derivatives thereof.

14. The device of claim 13 wherein said neurotransmitter comprises dopamine.

15. The device of claim 13 wherein said precursor comprises L-dopa.

16. The device of claim 1 wherein said active factor is selected from the group consisting of a growth factor and an active fragment, analog, and derivative thereof.

17. The device of claim 16 wherein said growth factor comprises fibroblast growth factor.

18. The device of claim 17 wherein said fibroblast growth factor comprises basic fibroblast growth factor.

19. The device of claim 18 wherein said fibroblast growth factor comprises acidic fibroblast growth factor.

20. The device of claim 1 wherein said active factor is selected from the group consisting of a hormone and an active fragment, analog, and derivative thereof.

21. A method for enhancing the secretion of an active factor from an active-factor secreting cell, said method comprising the steps of:

encapsulating said active factor-secreting cell in a semipermeable, electrically charged membrane; and

allowing said membrane to generate an electric surface charge, said charge causing said encapsulated cell to become depolarized, thereby inducing the secretion of said active factor therefrom.

22. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, membrane, said membrane being permeable to molecules having a molecular weight of about 50,000 daltons or less.

23. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, electret membrane, said membrane having a substantially tubular shape.

24. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a tube having a diameter of about 200 - 600 mm.

25. The device of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a membrane having a wall thickness ranging from about 50 - 100 mm.

26. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, piezoelectric membrane, and

said allowing step further comprises subjecting said piezoelectric membrane to mechanical stress, said stressed membrane generating a transient electric surface charge, said charge causing said encapsulated cell to become depolarized, thereby inducing the secretion of said active factor therefrom.

27. The device of claim 26 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, piezoelectric membrane, said membrane including a polyvinylidene difluoride-trifluroroethylene copolymer.

28. The method of claim 21 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable membrane, said membrane including a conductive polymer.

29. A method for providing an active factor to a target region of the body, said method comprising the steps of:

encapsulating an active factor-secreting cell in a semipermeable, electret membrane, said membrane being permeable to said active factor;

implanting said membrane in said target region; and

allowing said electret membrane to generate an electric surface charge, said charge causing said encapsulated cell to become depolarized, thereby inducing the secretion of said active factor therefrom.

30. The method of claim 29 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, membrane, said membrane being permeable to molecules having a molecular weight of about 50,000 daltons or less.

31. The method of claim 29 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a semipermeable, electret membrane, said membrane having a substantially tubular shape.

32. The method of claim 31 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a tube having a diameter of about 200 - 600 mm.

33. The device of claim 29 wherein said encapsulating step further comprises encapsulating said active factor-secreting cell in a membrane having a wall thickness ranging from about 50 - 100 mm.

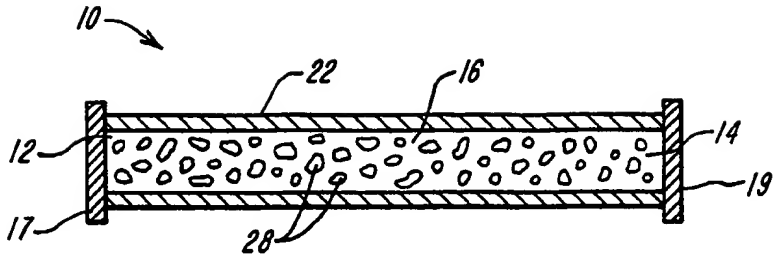

FIG. 1

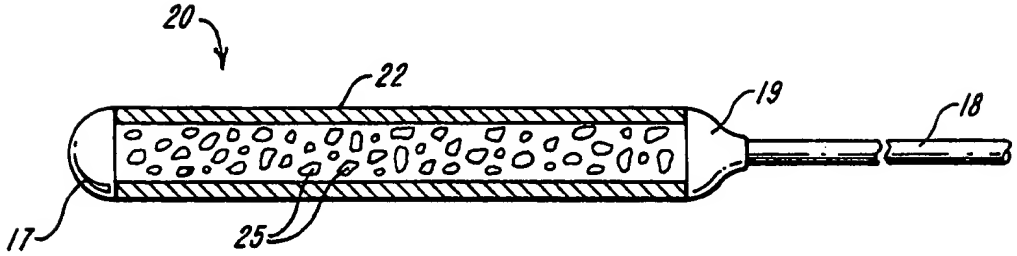

FIG. 2

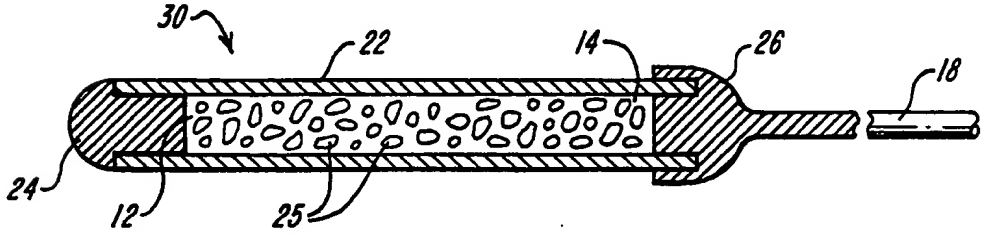

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application N T/US 91/00155

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 M 31/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 M, C 12 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A, 0127713 (GOOSEN) 12 December 1984 see abstract; page 4, lines 3-31; page 7, lines 3-34 --	1-6,11,21-25,28,29-33
A	US, A, 4364385 (LOSSEF) 21 December 1982 see abstract; column 3, lines 1-18; column 4, lines 1-16; figure 1 --	1-3,6,21-23,29-31
A	US, A, 4407957 (LIM) 4 October 1983 see abstract; claim 1 --	1-3,8,11
A	GB, A, 2094833 (DAMON) 22 September 1982 see claims 1,2 --	1,3,6

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25th April 1991	10. 06. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Danielle van der Haas

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP, A, 0274911 (PALL) 20 July 1988 see claim 1 --	8,10,11,27, 28
A	EP, A, 0290891 (SABEL) 17 November 1988 see claim 5 --	13-15
A	WO, A, 8912464 (MIT) 28 December 1989 see claim 1 --	16,17
P,A	WO, A, 9005552 (BROWN UNIVERSITY) 31 May 1990 see claims 1,4,5	16-19

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION

US 9100155
SA 44082

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0127713	12-12-84	CA-A- 1196862	19-11-85
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		US-A- 4673566	16-06-87
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		JP-A- 63180857	25-07-88
EP-A- 0290891	17-11-88	US-A- 4883666	28-11-89
WO-A- 8912464	28-12-89	None	

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9100155
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WO-A- 9005552	31-05-90	AU-A- 4620589	12-06-90

Docket No. A574

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A1

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(81) Designated States: CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: REDUCED FAT MEAT PRODUCT AND PROCESS OF MANUFACTURE

(57) Abstract

A reduced fat meat product is provided comprising meat and about 0.5-10 wt% oat beta-glucan amylodextrin, and a method of its manufacture. The meat is selected from any animal protein source and is preferably selected from the group consisting of beef, chicken, pork, lamb, veal, turkey and mixtures thereof.

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REDUCED FAT MEAT PRODUCT AND PROCESS OF MANUFACTURE

BACKGROUND OF THE INVENTION

This invention relates to a novel reduced fat meat product that plumps on cooking and which has improved texture and juiciness. The reduced fat meat product of the present invention incorporates oat beta-glucan amylodextrin.

Because of health concerns, reduced fat meat products are becoming increasingly popular. However, reduced fat meat products tend to have an organoleptically undesirable texture and are sometimes perceived as "dry" upon ingestion. Thus, a need presently exists for a reduced fat meat product with an improved texture and juiciness.

SUMMARY OF THE INVENTION

According to this invention a reduced fat meat product is provided comprising from about 0.5-10 wt% oat beta-glucan amylodextrin. Preferably, the meat product constitutes about 80-99 wt% meat wherein the meat has less than 12 wt% fat, about 0.5-5 wt% oat beta-glucan amylodextrin and about 0.5-15 wt% meat adjuncts. Most preferably, the reduced fat meat product of this invention comprises about 80-95 wt% meat, about 2-3 wt% oat beta-glucan amylodextrin and 0.5-10 wt% meat adjuncts.

In another aspect of the present invention, a method of processing a reduced fat meat product is provided wherein a quantity of meat is mixed with about 0.5-10 wt% oat beta-glucan amylodextrin. Preferably,

the reduced fat meat product is made by mixing about 80-99 wt% meat having less than about 12 wt% fat, 0.5-5 wt% oat beta-glucan amylodextrin and about 0.5-15 wt% meat adjuncts. Even more preferably, about 80-95 wt% meat having less than about 12 wt% fat, about 2-3 wt% oat beta-glucan amylodextrin and about 0.5-10 wt% meat adjuncts are mixed to make the reduced fat meat product of this invention. Most preferably, the oat beta-glucan amylodextrin and meat adjuncts are first dry mixed and hydrated with water prior to mixing with the meat.

As used herein the term "meat" refers to any animal protein source, such as beef, poultry, lamb, pork, veal, as well as processed meats such as hot dogs and sausages.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a flow diagram of a presently preferred process for making oat beta-glucan amylodextrin used in the process of FIG. 2.

FIG. 2 is a flow diagram of a presently preferred process for making the preferred embodiments of the meat product of this invention.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

It has unexpectedly been discovered that the reduced fat meat products of this invention comprising 0.5-10 wt% oat beta-glucan amylodextrin plump on cooking, and that these meat products further have improved texture and juiciness. Without intending to be restricted thereto, it is theorized that oat beta-glucan amylodextrin encapsulates available water in the meat. When subjected to cooking temperatures the oat beta-glucan amylodextrin expands to accommodate the greater volume occupied by the water vapor entrapped therein. This apparently causes the meat product to

plump on cooking. Furthermore, the entrapped moisture enhances the texture and juiciness of the low fat meat product.

All animal protein sources are contemplated for use in the reduced fat meat product of the present invention. Animal protein sources include but are not necessarily limited to beef, poultry, lamb, pork, veal, as well as processed meats such as hot dogs and sausages. Preferably, the meats are selected from the group consisting of beef, poultry, lamb, pork, veal and mixtures thereof. Even more preferred meats are selected from the group consisting of turkey, chicken and beef. The most preferred meat is beef. In a preferred embodiment of this invention, the meat will contain less than about 12 wt% fat. More preferably, the meat will contain less than about 9 wt% fat. Most preferably, the meat will comprise less than about 5 wt% fat.

According to the present invention, a reduced fat meat product is provided comprising from about 0.5-10 wt% oat beta-glucan amylodextrin. Preferably, oat beta-glucan amylodextrin comprises about 0.5-5 wt% of the reduced fat meat product, and even more preferably about 2-3 wt%.

Oat beta-glucan amylodextrin is a combination of maltodextrin and beta-glucan derived from the enzymatic treatment of oats. Oat beta-glucan amylodextrin may be prepared according to the method disclosed in U.S. Patent No. 4,996,063, incorporated herein by reference. Oat beta-glucan amylodextrin is known under the tradename OATRIM, and can vary in its beta-glucan content depending on the starting material. The beta-glucan content is identified by a number suffix. For example, OATRIM-5 contains about 4.5% - 5.5% by dry weight beta-glucan and is made from whole oat flour. OATRIM-10 contains about 9.5% - 10.5% by dry weight beta-glucan and is prepared from oat bran.

OATRIM-1 contains about 0.5% - 1.5% by dry weight beta-glucan and is prepared from debranned oats. Preferably, oat beta-glucan amylodextrin having about 4.5% - 5.5% by dry weight beta-glucan (OATRIM-5) is incorporated in the reduced fat meat product of the present invention.

The oat beta-glucan amylodextrin is preferably prepared according to the following process. With reference to FIG. 1, the first step in the illustrated process is to introduce 1,750 lbs. water, 339 grams calcium chloride (50 p.p.m.) and 250 lbs. whole oat flour (12.5% solids level) into a liquifier 12. A suitable whole oat flour is available from ConAgra Oat Mill, South Sioux City, NE. The pH of the mixture in the liquifier 12 should be within the range of 5.9 to 6.3. If necessary, the pH may be adjusted by the addition of sodium hydroxide or hydrogen chloride. The mixture in the liquifier 12 is intensely mixed for about ten minutes while simultaneously raising the temperature of the mixture to about 85°C. 272 ml of a thermo-stable alpha-amylase enzyme, which is available from Solvay Enzymes, Inc., Elkhart IN under the name TAKA-THERM L-340, is then added to the liquifier 12 and allowed to react with the mixture therein for about two minutes. The alpha-amylase is then deactivated by the addition of hydrogen chloride (5N) to the liquifier 12 to lower the pH to 4.5, and raising the temperature of the mixture in the liquifier 12 to 95°C.

The mixture in the liquifier 12 is then transferred via pump 14 to a holding tank 16. The holding tank 16 is provided with agitation, and the mixture is mixed well for about 10 minutes. The mixture in the holding tank 16 is then transferred via pump 18 through a triple tube cooler 20 where the mixture is quickly cooled to 40°C. The mixture is then transferred via pump 22 to a balance tank 24. The balance tank 24 is provided with agitation. The

mixture remains in the balance tank 24 only for a time sufficient for the entire mixture to be transferred to the balance tank 24.

The mixture in balance tank 24 is then immediately transferred via pump 26 to a centrifuge 28. The mixture is centrifuged and the solid phase of the mixture is discharged by gravity to a collector 30 and can be later sold as animal feed. The soluble stream from the centrifuge 28 is transferred by pump 32 to a balance tank 34. The balance tank 34 is provided with agitation. The soluble stream remains in the balance tank 34 only for a time sufficient for the entire soluble stream to be transferred to the balance tank 34. The soluble stream is then immediately transferred by pump 36 to a spray dryer 38 for drying. The resulting powder is then transferred by gravity from the spray dryer 38 to a 50 lb. bag 40 for shipment.

In addition to oat beta-glucan amylodextrin, the reduced fat meat products of this invention preferably contain meat adjuncts. Meat adjuncts are used to further enhance the organoleptic character of the reduced fat meat products of the present invention. Meat adjuncts include but are not necessarily limited to flavorings, spices, dietary fibers, binders, gums, flours and salts. Preferably, the meat adjuncts will comprise about 0.5-15 wt% of the reduced fat meat product of this invention. Even more preferably, the meat adjuncts will comprise about 0.5-10 wt% of the reduced fat meat product of this invention.

Flavorings may comprise about 0.1-2 wt% of the reduced fat meat product, and include but are not necessarily limited to beef stock, hydrolyzed vegetable proteins, autolyzed yeast, sodium lactate, beef plasma and the like. Spices may comprise about 0.1-4 wt% of the reduced fat meat product, and include but are not necessarily limited to dill, salt, peppers (black, red, green, pink, and the like), onion powder, garlic

powder, dextrose and the like. Dietary fibers may comprise about 0.1-2 wt% of the reduced fat meat product, and include but are not necessary limited to oat fiber, soy fiber, wheat fiber and pea fiber. Binders may comprise about 0.1-4 wt% of the reduced fat meat product, and include but are not necessarily limited to corn starch, potato starch, rice starch, tapioca starch, maltodextrin and the like. Preferred binders are corn starch, potato starch and maltodextrin. Gums may comprise about 0.1-1.0 wt% of the reduced fat meat product, and include but are not necessarily limited to carrageenan, guar gum, xanthan gum and locust bean gum. Flours may comprise about 0.5-5 wt% of the reduced fat meat product, and include but are not necessarily limited to oat flour, wheat flour, rice flour and tapioca flour. Salt may comprise about 0.1-2 wt% of the reduced fat meat product, and include but are not necessarily limited to table salt (sodium chloride), sodium citrate and potassium chloride. The salt may be flake, ingredient or encapsulated with hydrogenated vegetable oil. Where the total amount of dietary fiber and binder in the reduced fat meat product of this invention is below about 5 wt%, flake or ingredient salt is preferred. Conversely, where the reduced fat meat product contains about 5 wt% or more of dietary fiber and binder, encapsulated salt is presently preferred. Preferably, the meat adjuncts used in the reduced fat meat product of this invention are beef stock and table salt.

The oat beta-glucan amylodextrin may be mixed with the meat in either dry or hydrated form. However, the oat beta-glucan amylodextrin is preferably first hydrated prior to mixing with the meat. Specifically, the oat beta-glucan amylodextrin is first dry mixed with the optional meat adjuncts, and then this mixture is hydrated to form a non-meat slurry. This non-meat slurry is then mixed with the meat to yield the reduced

fat meat products of the present invention. Without being restricted thereto, it is theorized that the hydration of the oat beta-glucan amylodextrin enhances its ability to encapsulate water, thereby imparting greater plumping and improved texture and juiciness to the reduced fat meat product on cooking.

Turning now to FIG. 2, the first step in the illustrated preferred process is to introduce the meat into a grinder 12, and grind the meat once through a 5/8 inch plate. The resulting coarse ground meat is then augured into a ribbon blender 14 until the blender scale indicates that the desired quantity of meat has been introduced. Prior to blending, quality assurance personnel transfer a twenty pound sample of the meat from the ribbon blender 14 to a grinder 16, and fine grind the sample once through a 3/32 inch plate. The finely ground meat is tested with a fat analyzer 17 for compliance with batch fat specifications. The batch is adjusted where necessary to meet specifications.

Simultaneous to the above process, the aqueous non-meat slurry is prepared for addition to the meat. The first step is to introduce oat beta-glucan amylodextrin and any optional meat adjuncts into a blender 18. The oat beta-glucan amylodextrin and optional meat adjuncts are mixed for about 1-4 minutes until homogenous. A preweighed amount of water is then slowly added to the blender 18, and the resulting non-meat mixture is thoroughly mixed to yield an aqueous non-meat slurry. Preferably about 2-6 parts by weight water to 1 part by dry weight of the non-meat mix are added to the blender 18. More preferably, about 3-5 parts by weight water to 1 part dry weight of the non-meat mix are added, with the most preferred amount of water added being about 3.5 parts by weight water to 1 part dry weight of the non-meat mix. The resulting aqueous non-meat slurry is then transferred to the blender 14.

After insuring that the meat batch in the blender 14 conforms to the batch specification, the blender 14 is turned on and the meat and aqueous non-meat slurry are mixed for about 2-5 minutes in each direction to thoroughly disperse the non-meat slurry in the meat. A carbon dioxide injector 22 is utilized to inject carbon dioxide via an injection apparatus 23 into the meat mix in the blender 14 to maintain the meat temperature between about 28°F and 30°F.

The meat mix from the blender 14 is then augured to a grinder 24. The mix in the grinder 24 is then subjected to a final grind through a 1/8 inch plate.

If the finished product is to be fine ground chubs, the meat mix from the grinder 24 is augured through a ground meat pump 25 to a chub packaging machine 26. The meat mix in the chub packaging machine 26 is lined with plastic chub film and then transferred via a motorized conveyor to a chub chiller 38 where the temperature of the meat chub is reduced to about 24-25°F. From the chub chiller 38, the meat chubs are hand-packed for retail 40 or institutional 42 use.

If the finished product is to be formed into patties, the meat mix from the grinder 24 is augured to the holding tub 28. The mix from the tub 28 is then transferred by an elevator to a patty stamper 30. The meat patties are then transferred through a freezer 32 where the patty temperature is reduced to 0°F internal temperature. The meat patties are then packaged for retail 34 or institutional 36 use.

The reduced fat meat products of the present invention can be cooked according to any method as, for example, baking, frying, broiling or grilling. Suitable cooking temperatures range from about 275-475°F. Preferred cooking temperatures are in the range from about 300-375°F; the most preferred cooking temperature being in the range from about 325-350°F.

The reduced fat meat products of this invention may be prepared from rare doneness (internal meat temperature of about 140°F) to well doneness (internal meat temperature of about 160°F). However, best results (i.e. plumpness, texture and juiciness) are realized when the reduced fat meat product of this invention is cooked to medium-rare (internal meat temperature of about 145°F) or medium doneness (internal meat temperature of about 150°F).

EXAMPLES

In examples 1-6, five pound batches of reduced fat meat products were prepared according to the following formulations:

BATCH 1 (Beef)

Ingredient	wt%
Beef[1]	90
Aqueous non-meat slurry[2]	10
	100%

[1] Lean Beef Peeled Shank Meat Available from Monfort, Inc., Greely, CO (about 8-10 wt% fat).

[2] Containing 70 wt% water; 20 wt% oat beta-glucan amylodextrin; 6.5 wt% spray dried beef stock; and 3.5 wt% table salt.

BATCH 1a Control (Beef)

Ingredient	wt%
Beef[1]	100

[1] Lean Beef Peeled Shank Meat Available from Monfort, Inc., Greely, CO (about 8-10 wt% fat).

BATCH 2 (Turkey)

Ingredient	wt%
Turkey[1]	90
Aqueous non-meat slurry[2]	10
	100%

[1] 90 Lean Ground Turkey Available From Longmont Foods, Inc., Longmont, CO (about 10 wt% fat).

[2] Containing 70 wt% water; 20 wt% oat beta-glucan amylodextrin; 6.5 wt% spray dried beef stock; and 3.5 wt% table salt.

BATCH 2a Control (Turkey)

Ingredient	wt%
Turkey[1]	100

[1] 90 Lean Ground Turkey Available From Longmont Foods, Inc., Longmont CO (about 10 wt% fat).

BATCH 3 (Pork)

Ingredient	wt%
Pork[1]	90
Aqueous non-meat slurry[2]	10
	100%

[1] 90 Lean Ground Pork Available from Monfort, Inc., Worthington, MN (about 10 wt% fat).

[2] Containing 70 wt% water; 20 wt% oat beta-glucan amylodextrin; 6.5 wt% spray dried beef stock; and 3.5 wt% table salt.

BATCH 3a Control Pork)

Ingredient	wt%
Pork[1]	100%

[1] 90 Lean Ground Pork Available From Monfort, Inc., Worthington, MN (about 10 wt% fat).

The meat batches having the indicated formulations were prepared according to a method similar to that previously described, the primary difference being that the meat patties used in these examples were prepared on a smaller scale. The control batches were prepared by grinding the meat in a Butcher Boy 3/4 horsepower grinder through a 3/32 inch plate. Nothing was added to the control meat batches. The meat products of this invention were prepared by first dry mixing by hand the oat beta-glucan amylodextrin, spray dried beef stock and table salt. The water was then slowly added to this dry mixture to make an aqueous non-meat slurry. The resulting aqueous non-meat slurry was then uniformly dispersed in the meat by hand, the meat having been previously ground in a Butcher Boy 3/4 horsepower grinder through a 3/32 inch plate. Four ounce patties were then prepared by hand from the meat batches. The patties were about 18 mm in height and about 95 mm in diameter. These meat patties were cooked to various degrees of doneness and according to various methods. The amount by which these meat patties plumped on cooking was measured as the increase in height in the center of the patty. These meat patties were compared to controls for plumpness.

Example 1

Table 3 relates to a measure of the plumpness of turkey and pork products of the present invention. Specifically, turkey patties and pork patties of this invention were compared to control turkey and pork patties. The patties were pan-fried to medium doneness (about 150°F internal meat temperature), and measured for their plumpness. Table 3 shows that turkey and pork patties increased in height by an amount more than twice that of the controls on cooking.

Example 2

Table 4 relates to a measure of the plumpness on cooking of a beef product of the present invention. Beef patties of the present invention were compared to control beef patties. The patties were pan-fried to medium doneness, and measured for their plumpness. Table 4 shows that the beef patties of the present invention increased in height by an amount almost three times greater than compared with the controls on cooking.

Example 3

Table 5 demonstrates that a beef product of the present invention increased by about one-third more in height if pan-fried fresh than if pan-fried from an initially frozen state.

Example 4

Table 6 shows beef products of the present invention cooked to various degrees of doneness. As demonstrated by Table 6, beef patties pan-fried to medium rare and medium doneness increased in height on cooking about twice as much as beef patties cooked to rare or well doneness.

Example 5

Table 7 shows beef products of the present invention grilled and pan-fried to medium doneness. As demonstrated by Table 7, pan-fried beef patties experienced a greater increase in height on cooking than did grilled beef patties.

Example 6

Table 8 shows beef products of the present invention pan-fried to medium doneness. As demonstrated by Table 8, the beef patties retain substantially all of their plumpness on standing for ten minutes.

Example 7

Table 9 demonstrates that a beef product of the present invention retains 2.82% more moisture on cooking than a control beef patty. This increased moisture retention results in a beef product which has better texture and improved juiciness.

Example 8

Beef, turkey and pork products of this invention were prepared and evaluated for overall taste as compared to control products.

The control beef and pork products were made by first grinding the meat in a Hobart Grinder through a 1/2" plate, and then through a 1/8" plate. The turkey was obtained already ground from Longmont Foods, Longmont, CO. The ground beef, pork and turkey were made into 4 oz. patties, 4" in diameter and 1/2" thick using a Hollymatic Model No. 54 patty machine. During the aforesaid process, the meat was maintained at 30°F. The patties were then blast frozen to -10°F using a mechanical blast freezer and kept for about ten days until cooking for sensory evaluation.

A non-meat slurry comprising 70% water, 20 wt% oat beta-glucan amylodextrin, 6.5 wt% spray dried beef stock and 3.5 wt% table salt was prepared by first dry mixing the oat beta-glucan amylodextrin, spray dried beef stock and table salt. This mixture was then hydrated with the water in a stainless steel bowl using a wire whip to slurry the ingredients. The beef and pork patties of this invention were prepared by

grinding the meat in a Hobart Grinder through a 1/2" plate. Again, the turkey was obtained from Longmont Foods already ground. 90 parts of the ground beef, pork and turkey were separately placed in a Keebler vacuum mixer and mixed with 10 parts of the non-meat slurry. A vacuum was applied at 26 inches and the mixture was allowed to mix for 3 minutes. The vacuum was removed and the temperature of the mix was reduced to 28-30°F for beef and pork and 26-28°F for turkey by the addition of CO_2 pellets and further mixing for 2-4 minutes. The mixture was then transferred to a Hobart Grinder and ground through an 1/8 inch plate. The meat was then stamped into patties and frozen as described with respect to the controls.

As Table 10 demonstrates, the reduced fat turkey and beef products of the present invention manifested overall organoleptic acceptability superior to control patties. The pork products of the present invention exhibited overall organoleptic acceptability about the same as the control patties.

Of course, it should be understood that a wide range of changes and modifications can be made to the preferred embodiments described above. This invention is believed applicable to the widest variety of animal protein sources. It is therefore intended that the foregoing detailed description be regarded as illustrative rather than limiting, and that it be understood that it is the following claims, including all equivalents, which are intended to define the scope of this invention.

TABLE 1 (FIG. 1)

Reference No.	Description	Identification
12	Liquifier	BREDDO LIQUIFIER Model No. 52E (250 Gal.)
14	Pump	TRICLOVER PUMP Model No. CP-25 (150 Gal./minute)
16	Holding Tank	MUELLER 600 Gallon Tank
18	Pump	TRICLOVER PUMP Model No. CP-25 (150 Gal./minute)
20	Triple Tube Cooler	TRIPLETUBE COOLER (2" OD x 1.5" ID x 268 feet length)
22	Pump	TRICLOVER PUMP Model No. CP-25 (150 Gal./minute)
24	Balance Tank	MUELLER 1200 Gallon Tank
26	Pump	TRICLOVER PUMP Model No. CP-25 (150 Gal./minute)
28	Centrifuge	ALFA-LAVAL/SHARPES Centrifuge L Model No. P-3400 HHS
30	Collector	55 gallon drum
32	Pump	TRICLOVER PUMP Model No. CP-25 (150 gal./minute)
34	Balance Tank	MUELLER 600 Gallon Tank
36	Pump	TRICLOVER PUMP Model No. CP-25 (150 Gal./minute)
38	Spray Dryer	COULTER SPRAY DRYER (Evaporates 2000 lb. water/hr.)
40	50 lb. bag	

TABLE 2 (FIG. 2)

Reference No.	Description	Identification
12	Grinder	WEILER grinder Model No. 1109B, 5/8 inch plate
14	Blender	R.M.F. Ribbon Blender MEPAC 170, Model No. MB8000
16	Grinder	HOBART grinder, 3/32 inch plate
17	Fat Analyzer	KARTRIDGE PAK Corp. Anal-Ray Model No. 316-3/M-201
18	Blender	HOBART mixer
22	CO_2 Injector	Pressurized CO_2
23	Injection Apparatus	Ten CARDOX VALVES Model No. 133ADA
24	Grinder	WEILER grinder, Model No. 1109B 1/8 inch plate
25	Ground Meat Pump	VEMAG Model No. DC-3000
26	Chub Packaging Machine	KARTRIDGE PAK Corp.
28	Tub	500 lb. Stainless Steel Tub
30	Patty Stamper	FORMAX F28
32	Freezer	ROSS IQF Freezer
34	Retail Packaging	Cardboard Box Polybagged-12 patties/box
36	Institutional Packaging	Cardboard Box Polybagged-80 patties/box
38	Chub Chiller	MYER METALCRAFT Propylene Glycol Chub Chiller Model No. 2-12M
40	Retail Packaging	Cardboard Box Polybagged-12 patties/box
42	Institutional Packaging	Cardboard box Polybagged-80 patties/box

TABLE 3

Pan Fry, 325°F, Medium Doneness

	Diameter Avg. (%Δ)	Height Avg. (%Δ)	Yield Avg.(%Δ)[5]
BATCH 2 (Turkey)[1]	-26.2	+45.6	-28
BATCH 2a (Control)[2]	-23.9	+22.0	-35.2
BATCH 3 (Pork)[3]	-22.2	+26.9	-23.3
BATCH 3a (Control)[4]	-20.1	+10.1	-26.5

[1] Eight patties were cooked and measured.
[2] Four patties were cooked and measured.
[3] Eight patties were cooked and measured.
[4] Four patties were cooked and measured.
[5] Change in weight of the patties on cooking was determined by weighing the patty just prior to and immediately after cooking.

TABLE 4

Pan Fry, 350°F, Medium Doneness

	Diameter Avg. (%Δ)	Height Avg. (%Δ)	Yield Avg.(%Δ)
BATCH 1 (Beef)[1]	-23.2	+56.0	-22.0
BATCH 1a (Control)[2]	-18.4	+19.7	-24.2

[1] Four patties were cooked and measured.
[2] Four patties were cooked and measured.

TABLE 5

Pan Fry, 350°F, Medium Doneness

	Diameter Avg. (%Δ)	Height Avg. (%Δ)	Yield Avg.(%Δ)
BATCH 1 (Beef) FRESH[1]	-24.0	+62.3	-26.7
BATCH 1 (Beef) FROZEN[2]	-23.9	+41.4	-26.9

[1] Six patties were cooked and measured; "Fresh" - about 35°F.
[2] Six patties were cooked and measured; "Frozen" -about 0°F.

TABLE 6

Pan Fry, 325°F

	Diameter Avg. (%)	Height Avg. (%)	Yield Avg.(%)
BATCH 1 (Beef)- Rare Doneness[1]	-20.3	+21.6	-15.2
BATCH 1 (Beef) - Medium Rare Doneness[2]	-25.0	+46.5	-22.7
BATCH 1 (Beef)- Medium Doneness[3]	-24.5	+42.3	-26.7
BATCH 1 (Beef)- Well Doneness[4]	-21.9	+23.2	-34.1

[1] Six patties were cooked and measured; Internal Meat Temp. about 140°F.
[2] Six patties were cooked and measured; Internal Meat Temp. about 145°F.
[3] Six patties were cooked and measured; Internal Meat Temp. about 150°F.
[4] Six patties were cooked and measured; Internal Meat Temp. about 160°F.

TABLE 7

350°F, Medium Doneness

	Diameter Avg. (%Δ)	Height Avg. (%Δ)	Yield Avg.(%Δ)
BATCH 1 (Beef)- Pan Fry[1]	-20.9	+34.0	-27.0
BATCH 1 (Beef)- Grilled[2]	-21.2	+29.9	-25.5

[1] Six patties cooked from frozen and measured.
[2] Six patties cooked from frozen and measured.

TABLE 8

BATCH 1 (Beef), Pan Fry 350°F, Medium Doneness[1]

Time (Seconds)	Height Avg. (Δ in mm)	Avg. Height Change (%Δ)
Raw	17.9	-
0	26.7	+49.2
60	25.4	-4.9
120	25.2	-0.8
210	24.8	-1.6
300	24.5	-1.2
600	23.6	-3.7

[1] Six patties cooked from frozen and measured.

TABLE 9

	Avg. Fat (wt%)	Avg. Moisture (wt%)	Avg. Moisture ((%Δ)
BATCH 1 (Beef)-Raw[1]	9.88	69.17	-9.28
BATCH 1 (Beef)-Cooked [2]	11.12	62.76	
BATCH 1a (Control)-Raw[1]	10.63	69.57	
BATCH 1a (Control)-Cooked[3]	11.42	61.15	-12.1

[1] One patty of each was measured
[2] Four patties were pan-fried to medium doneness and measured.
[3] Four patties were pan-fried to medium doneness and measured.

Sensory Evaluation

TABLE 10

	Overall Acceptance[1]
Beef Patties	
Test	7.4
Control	6.1
Pork Patties	
Test	5.7
Control	5.6
Turkey Patties	
Test	6.8
Control	5.8

[1] Test Method: A nine-point hedonic scale converted to a nine-point rating scale; 1=dislike extremely; 5=neither like nor dislike; 9=like extremely. Seventy-six Subjects (forty males, thirty six females) randomly selected from consumers of hamburger/meat patties, sausage patties, or chicken/turkey bread patties. The patties were heated in an electric skillet over medium heat for 14-16 minutes, flipping once. Turkey patties cooked to an internal temperature of 170°F, beef patties cooked to an internal temperature of 160°F and pork patties cooked to an internal temperature of 180°F. The meat patties were served on six-inch white styrofoam plates on a white service tray. Each subject tasted all six products according to balanced block, sequential monodic method. One minute between samples with a five minute break between types of products (i.e. beef, pork, and turkey). Distilled water was used as the rinse water and an unsalted cracker as the mouth cleanser. Lighting consisted of a 40 watt, 115-125 volt GE Lumiline bulb with a soft white diffuser in each booth.

WE CLAIM:

1. A meat product comprising about 0.5-10 wt% oat beta-glucan amylodextrin.

2. The meat product of claim 1 comprising less than about 12 wt% fat.

3. The meat product of claim 2 wherein the meat is selected from the group consisting of beef, turkey, chicken, lamb, pork, veal and mixtures thereof.

4. The meat product of claim 2 wherein the meat comprises beef.

5. A meat product comprising:

about 80-99 wt% meat having less than about 12 wt% fat;

about 0.5-10 wt% oat beta-glucan amylodextrin; and

about 0.5-15 wt% meat adjuncts.

6. The meat product of claim 5 wherein the meat is selected from the group consisting of beef, turkey, chicken, lamb, pork, veal and mixtures thereof.

7. The meat product of claim 6 comprising about 0.5-5 wt% oat beta-glucan amylodextrin.

8. The meat product of claim 6 comprising about 2-3 wt% oat beta-glucan amylodextrin.

9. The meat product of claim 8 wherein the meat comprises beef.

10. A meat product comprising:

about 80-99 wt% beef having less than 12 wt% fat;

about 0.5-5 wt% oat beta-glucan amylodextrin; and

about 0.5-15 wt% meat adjuncts.

11. A method of manufacturing a meat product comprising mixing about 0.5-10 wt% oat beta-glucan with a quantity of meat.

12. The method of claim 11 wherein the oat beta-glucan amylodextrin is hydrated prior to mixing with the meat.

13. The meat product made by the method of claim 11 wherein the quantity of meat comprises less than about 12 wt% fat.

14. The method of claim 13 wherein the meat is selected from the group consisting of beef, turkey, chicken, lamb, pork, veal and mixtures thereof.

15. The method of claim 13 wherein the meat comprises beef.

16. A method of manufacturing a meat product comprising mixing:

about 80-99 wt% beef;

about 0.5-5 wt% of oat beta-glucan amylodextrin; and

about 0.5-15 wt% meat adjuncts.

17. The method of claim 16 wherein the oat beta-glucan amylodextrin is hydrated prior to mixing with the meat.

18. The meat product made by the method of claim 17 wherein the meat comprises less than about 12 wt% fat.

19. A method of manufacturing a meat product comprising:

providing a quantity of an aqueous non-meat slurry comprising oat beta-glucan amylodextrin and meat adjuncts; and

mixing a quantity of the aqueous non-meat slurry with a quantity of meat having less than about 12 wt% fat such that the resulting meat product comprises about 80-99 wt% meat, about 0.5-10 wt% oat beta-glucan amylodextrin and about 0.5-15 wt% meat adjuncts.

20. The method of claim 19 wherein the meat is selected from the group consisting of beef, chicken, turkey, pork, veal, lamb and mixtures thereof.

21. The method of claim 20 wherein the meat product is stamped into a patty.

22. The method of claim 20 wherein the meat product is formed into a chub.

23. A meat patty comprising:

about 80-99 wt% meat having less than 12 wt% fat;

about 0.5-10 wt% oat beta-glucan amylodextrin; and

about 0.5-15 wt% meat adjuncts.

24. The meat patty of claim 23 wherein the meat is selected from the group consisting of beef, turkey, chicken, veal, pork, lamb and mixtures thereof.

25. A meat chub comprising:

about 80-99 wt% meat having less than about 12 wt% fat;

about 0.5-10 wt% oat beta-glucan amylodextrin; and

about 0.5-15 wt% meat adjuncts.

26. The meat chub of claim 25 wherein the meat is selected from the group consisting of beef, turkey, chicken, veal, pork, lamb and mixtures thereof.

27. A meat product comprising a quantity of chicken and about 0.5-10 wt% oat beta-glucan amylodextrin.

28. The meat product of claim 27 further comprising about 0.5-15 wt% meat adjuncts.

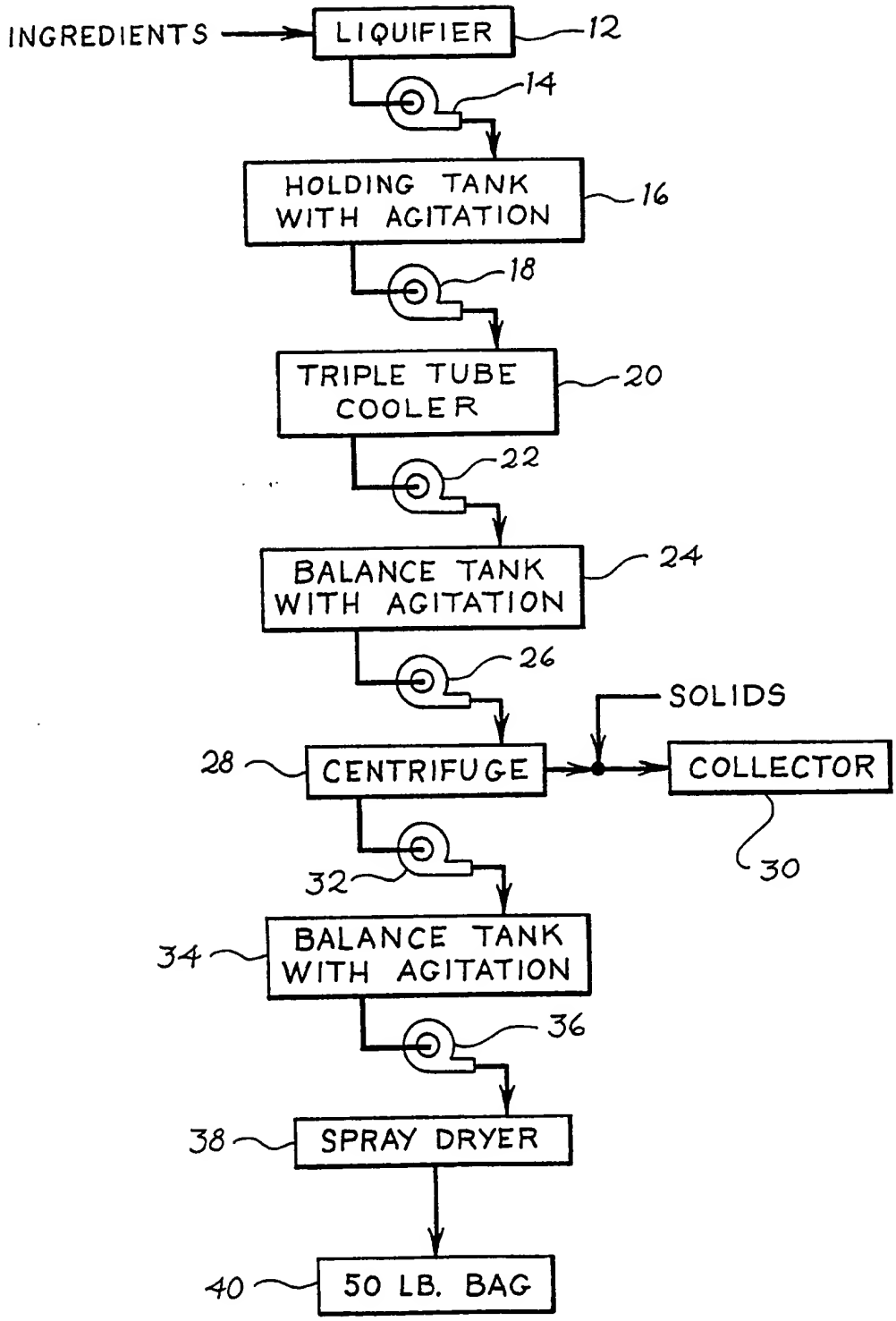
29. A meat product comprising:

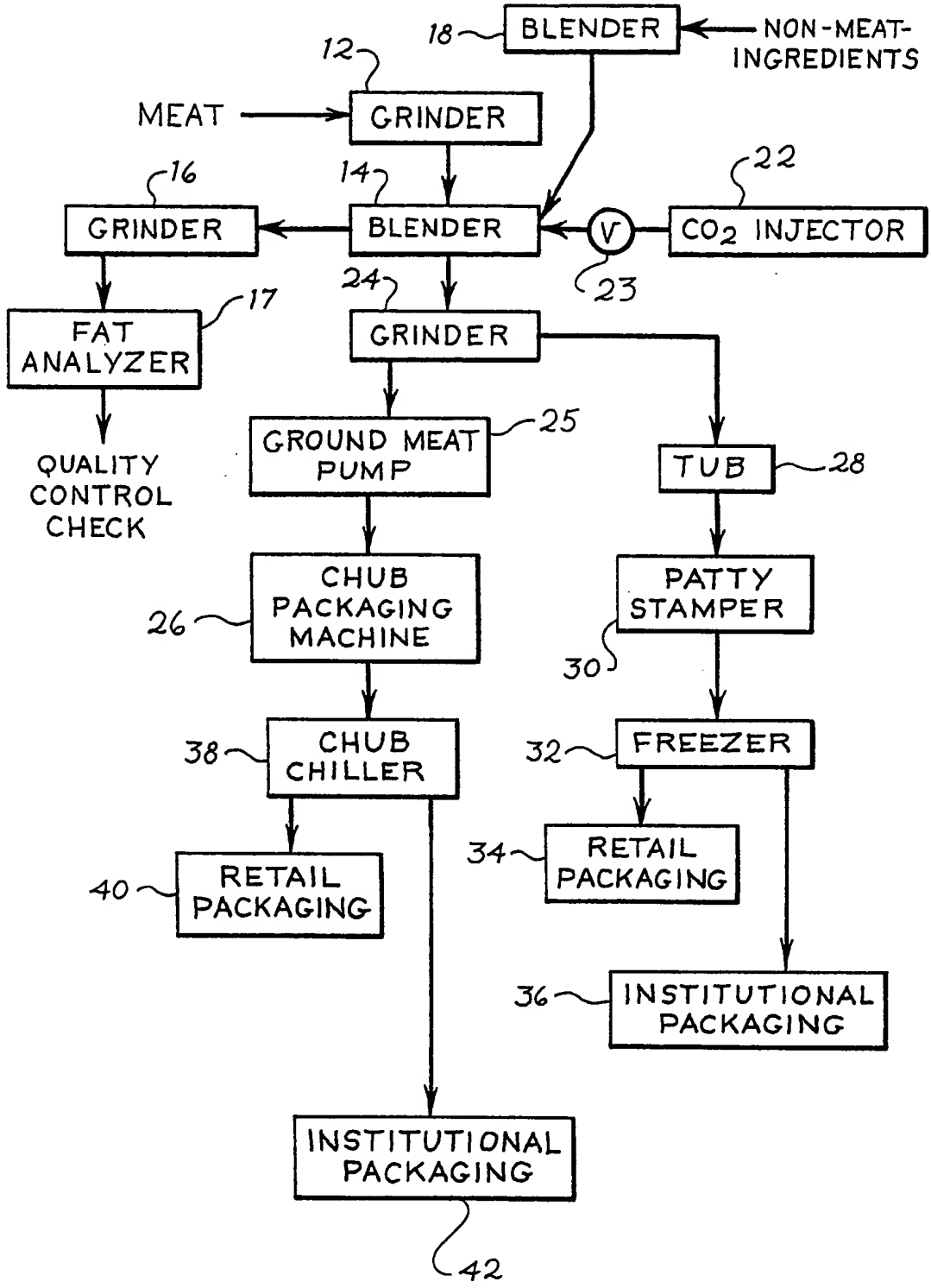
about 80-99 wt% meat having less than about 5 wt% fat;

about 0.5-10 wt% oat beta-glucan amylodextrin; and

about 0.5-15 wt% meat adjuncts.

30. The meat product of claim 29 wherein the meat is selected from the group consisting of beef, turkey, chicken, lamb, pork, veal and mixtures thereof.


INGREDIENTS
LIQUIFIER
12
14
HOLDING TANK WITH AGITATION
16
18
TRIPLE TUBE COOLER
20
22
BALANCE TANK WITH AGITATION
24
26
SOLIDS
28
CENTRIFUGE
COLLECTOR
30
32
34
BALANCE TANK WITH AGITATION
36
38
SPRAY DRYER
40
50 LB. BAG


18
BLENDER
NON-MEAT-INGREDIENTS
12
MEAT
GRINDER
16
14
22
GRINDER
BLENDER
V
CO_2 INJECTOR
23
24
17
FAT ANALYZER
GRINDER
QUALITY CONTROL CHECK
GROUND MEAT PUMP
25
TUB
28
CHUB PACKAGING MACHINE
26
PATTY STAMPER
30
38
CHUB CHILLER
32
FREEZER
34
RETAIL PACKAGING
40
RETAIL PACKAGING
36
INSTITUTIONAL PACKAGING
INSTITUTIONAL PACKAGING
42

INTERNATIONAL SEARCH REPORT

International application No.
P[redacted]92/05613

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A23L 1/314
US CL :426/646
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/641, 644

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	USDA, June 1991, Inglett et al, "Oatrim: New Maltodextrim Fat Substitute," pages 1-4.	1-30
Y	Food Processing, August 1990. , Duxbury, "Oatrim Fat Reducer, Cholesterol Fighter," page 1-3.	1-30
Y	USDA News Release, April 1990, "Cholesterol-Fighting Fiber Cuts Fat, Calories in Ice Cream," pages 1-3.	1-30
Y	Alabama Agricultural Experiment Station, March 1990, Frobish, "Advances in Lean Ground Beef Production," pages 1-27.	1-30
Y	US, A, 4,818,557 (CONRAD) 04 April 1989, See entire document.	1-30
Y	Webb Technical Group, Inc., November 1990, "Development of Low Fat Beef Patties with Added Dietary Fibers," pages 1-36.	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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